RISK ASSESSMENT FOR *LISTERIA MONOCYTOGENES* IN TRADITIONALLY PROCESSED FISH FROM INFORMAL MARKETS IN ACCRA AND TEMA

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BY

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DECLARATION

I hereby declare that I carried out this research under supervision in the Department of Nutrition and Food Science, University of Ghana, Legon. Works referred to have been dully acknowledged.

DEDICATION

To Professor Kwaku Tano-Debrah, for the blessing you have been.

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ABSTRACT

Traditionally processed fish contributes significantly to food and nutrition security in Ghana. The processing and handling has however been associated with unsanitary and unhygienic practices with documented occurrence of food-borne pathogens. The products are also mainly sold on informal markets, where earlier studies reported the occurrence of *Listeria monocytogenes* in products sold therein. This study sought to determine the occurrence of *L. monocytogenes* in traditionally processed (smoked, dried, salted) fish sold on informal markets and to assess the exposure of consumers to the pathogen and the associated risk of illness. The study was based on the Codex Alimentarius protocol for microbial risk assessment. Surveys were conducted on selected traditional processors and consumers to determine processing practices and consumption patterns (frequency and portion sizes) respectively. Samples of traditionally processed fish were procured from some processors and consumer markets in the survey locations for microbial analysis to determine the occurrence and concentrations of *L. monocytogenes* in the processed fish. Microbial challenge tests were also done by cooking deliberately-contaminated fish for short and long time intervals to determine the survival of the pathogen during domestic cooking. Data from the survey (quantities of fish often consumed) and the laboratory analyses (microbial load) were used to assess the exposure of consumers to the pathogen, and also fitted to parametric (probability) functions to characterize the dose response using Monte Carlo simulations with the @Risk software (version 5.5, Palisade Corporation). Prevalence of *L. monocytogenes* in the fish products sampled from the markets was high (40-80%). However, the pathogen was not detected in smoked fish sampled immediately after processing, suggesting that post-processing contamination occurred. The concentrations of the pathogen in the products were generally low

(10^{2-3} CFU/g), and decreased from smoked fish through to sundried fish. The pathogen also survived in fish used for the challenge test. The estimated risks of illness were low, ranging from 1 in 100 to 1 in 100,000,000,000 chances of illness. Higher risks of illness were recorded for consumption of smoked fish than for sundried fish and salted fish, in that order. Consumers with high susceptibility to *L. monocytogenes* infection (elderly, children and pregnant women) were at a greater risk of illness than low risk individuals (non-pregnant adults aged 18 – 39 years). The findings suggest that consumers are exposed to ingesting *L. monocytogenes* through consumption of traditionally processed fish on informal markets. However the risk of illness is low. Improvements in hygienic processing and post-processing handling of fish as well as proper cooking of the fish products before consumption are recommended.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Traditional fish processing is an enterprise that contributes significantly to efforts at ensuring food and nutrition security in Ghana. In coastal communities, the practice serves not only as a major economic activity for both men and women, but also ensures a continuous supply of their main source of animal protein - fish. Indeed, the entire country is known to consume fish in large quantities; per capita consumption in 2008 was close to twice the world average (Bank of Ghana, 2008). Much of the fish consumed in the country is traditionally processed (smoked, dried, fried, salted and/or fermented) (Nketsia-Tabiri and Sefa-Dedeh, 2000; Adu-Gyamfi, 2006).

Although simple and generally inexpensive, traditional fish processing has been characterized by poor quality control and unhygienic processing conditions that compromise the safety of the products (Sefa-Dedeh, 1989; 1993; Nketsia-Tabiri and Sefa-Dedeh, 2000). Additionally, most traditional fish processors sell their products on informal markets. These markets contribute to food and nutrition security by offering physical access to foods at low cost to a majority of Ghanaians. However, studies have shown that foods are generally not handled hygienically in these markets and therefore record high microbial counts. Oppey (2002), Cofie (2003) and Adu-Gyamfi (2006) found that smoked fish sold on such markets in Accra had high counts of coliforms, *Staphylococcus aureus*, *Escherichia coli* and other pathogens. It is therefore possible that food-borne illnesses such as staphylococcal poisoning by *S. aureus*, could result from consumption of the products. Additionally, illnesses could result from the presence of other pathogens such as *Listeria monocytogenes* known to

be associated with fish and introduced either during processing or post-processing handling.

1.2 *Listeria monocytogenes*

L. monocytogenes is a Gram-positive, catalase positive, non-spore forming food-borne bacterial pathogen responsible for a highly fatal disease called listeriosis. It is reported to cause an estimated 2,500 illnesses and 500 deaths annually in the United States of America alone (CDC, 2009), and is also considered the leading cause of death among food-borne bacterial pathogens, recording very high fatality:case ratios (Montville and Matthews, 2005; Jay, 2003).

The organism is widely distributed in the environment (ubiquitous), has long survival periods in foods, grows under very low temperatures (-1.5 ^{o}C), tolerates high salinity (up to 10 – 12% NaCl), low pH (minimum 4.4), and low water activity (minimum 0.83) (Farber and Peterkin, 1991; Garbutt, 1997; Sutherland and Porritt, 1997; Jay 2003; Montville and Matthews, 2005). As a result of these unique properties, it has the potential to easily contaminate food and, when it does, to survive traditional methods employed to prevent microbial growth in foods, such as reduction in water activity, storage under cold temperatures, salting and acidification (Montville and Matthews, 2005).

When ingested, *L. monocytogenes* has the unique ability to enter and grow in human phagocytes, thereby bypassing the inherent defensive mechanisms of the circulatory system (Montville and Matthews, 2005). Another special feature of this pathogen is that it has an uncommonly long incubation period (the time between ingestion of the

pathogen and the appearance of the first symptom of disease), reported to be typically between 1 – 70 days (Garbutt, 1997).

Listeriosis, the disease the organism causes, comes with adverse health conditions such as meningitis, encephalitis, corneal ulcer, pneumonia, and septicaemia (Jay 2003). In pregnant women, intrauterine or cervical infections could occur and subsequently lead to spontaneous abortion, pre-mature birth, still birth or prenatal sepsis that could cause neonatal meningitis or death of newborns within a week. It is estimated that generally, 20 – 30% of listeriosis victims die (Montville and Matthews, 2005).

1.3 *Listeria monocytogenes* and food safety

There has been an increasing global concern about *L. monocytogenes* and its influence on food safety. Several outbreaks of listeriosis have been reported in different parts of the world, with high fatalities. In 1998, a listeriosis occurrence in Finland resulted in 25 illnesses, of which only one person survived. The implicated food was butter. In France, 31 out of 32 infected persons died when an outbreak occurred in the year 2000 through consumption of pork contaminated with the pathogen. Similarly, when a listeriosis outbreak occurred in Canada in 2009 through red meat consumption, close to 50% of the infected persons (20 out of 53 cases) died (CDC, 2009). In September 2011, a listeriosis outbreak claimed 16 lives in 18 states in the USA (CDC, 2011). Cantaloupes from an eastern Colorado farm were implicated. These statistics clearly illustrate the food safety significance of *L. monocytogenes.* They also show that outbreaks of listeriosis, although sporadic, record very high fatalities (Table 1.1).

Table 1.1: Annual outbreaks of food-borne illnesses from selected pathogens

Pathogen	Cases	Illnesses	Deaths	% Deaths
Campylobacter spp.	1,963,141	10,539	99	0.95
Mackerelella non-typhoidal	1,341,873	15,608	553	3.54
E. coli O157:H7	62,458	1,843	52	2.82
E. coli non-O157-STEC	31,229	921	26	2.82
L. monocytogenes	**2,493**	**2,298**	**499**	**21.71**

Source: (CDC, 2009) Data true for USA

The pathogen is largely associated with foods such as ready-to-eat (RTE) meat products, milk and milk products, coleslaw, and fish (particularly vacuum packed and cold-smoked fish) (Jay, 2003; Adam and Moss, 2008).

1.4 Fish as a vehicle for *Listeria monocytogenes* transmission

Studies have shown that fish and fish products are suitable vehicles for the transmission of *L. monocytogenes*. The pathogen is frequently associated with RTE and heat-treated fishery products (Buchanan *et al.,* 1997; Okutani *et al.*, 2004; Basti *et al*., 2006). It has been isolated in smoked, salted, and salted and fermented fish (FAO, 1999).

The frequent occurrence of *L. monocytogenes* in fishery products raises a food safety concern since most of such products are not (adequately) heat treated further before consumption. There is therefore the possibility that the pathogen could survive and/or grow in the foods as they move along the distribution chain (FAO, 1999). Three outbreaks of listeriosis that occurred in the 1990s were traced to fish products (FAO,

1999). These demonstrate that fish and fish products are suitable vehicles for the transmission of the pathogen to humans.

1.5 Problem statement

Recent studies suggest the occurrence of *L. monocytogenes* in some foods in Ghana (Appiah, 2010; Dobge, 2010). Prior to these studies, published data on the occurrence of the pathogen in foods in Ghana were unavailable. Accordingly, as at 2010, specific national regulatory provisions for the control of the pathogen in Ghana were non-existent.

Secondly, fish has been recognized as a vehicle for the transmission of *L. monocytogenes*. Fish processed by smoking and salting, methods notably employed in Ghanaian traditional fish processing (Adu-Gyamfi, 2006), are of particular interest in this regard (Lindqvist and Westoo, 2000). Interestingly, about 80% of fish landed in Ghana is processed this way (Adu-Gyamfi, 2006; Nketstia-Tabiri and Sefa-Dedeh, 2000).

The lack of quality control in traditional fish processing makes contamination of the products highly probable. The processed fish are also sold mainly on informal markets where foods are generally sold under unsatisfactory hygienic conditions. Contamination with *L. monocytogenes* is therefore probable.

Fish also constitutes the bulk of animal protein in diets in Ghana, contributing 60% of the total animal protein in diets throughout the country (Steiner-Asiedu *et al*., 1991;

Plahar *et al*., 1991). Moreover, fish purchases are estimated to account for 22.4% of household food expenditures (BoG, 2008).

Given that more than 80% of the country's fish landings are traditionally processed, by extension, the country also consumes substantial quantities of traditionally processed fish. A food safety implication of this is that any compromise on the safety of these products could result in potentially devastating outbreaks of preventable food-borne illnesses.

1.6 Rationale for the study

The high consumption rates of (traditionally processed) fish in the country and the suitability of the products as vehicles for the transmission of *L. monocytogenes*, suggest that uncontrolled occurrence of the pathogen in the products could result in outbreaks of listeriosis. Beside its effect on the health of consumers, such contaminations could result in intangible economic losses through loss of consumer confidence in Ghanaian fishery products.

It was therefore important to conduct a situational analysis on the extent of contamination of traditionally processed fish sold on informal markets with *L. monocytogenes*, and to evaluate the exposure of consumers to the pathogen, hence this study.

The key research questions the study sought to answer were:

i. Is the occurrence of *L. monocytogenes* in traditionally processed fish a food safety issue in the consumption of the products?

ii. To what extent are consumers likely to ingest the pathogen through consumption of traditionally processed fish sold on informal markets?

1.7 Study objectives

1.7.1 Main objective

To determine the occurrence (presence and concentration) of *Listeria monocytogenes* in traditionally processed fish in Ghana, and to estimate the risk of ingesting the pathogen through consumption of traditionally processed fish.

1.7.2 Specific objectives

1. To assess the frequency and quantities of consumption of hot-smoked fish (mackerel, tuna and herrings), salted-dried tilapia (*koobi*), salted and fermented fish (*momoni*), chunks of salted and dried ray fish (*kako*), and sundried sardines among consumers in James Town and Tema New Town

2. To detect the presence and concentration of *Listeria monocytogenes* in the selected traditionally processed fish purchased from some informal markets in Accra and Tema

3. To detect the presence and concentration of *Listeria monocytogenes* in fish during traditional processing (from raw materials to finished products) and compare the findings with those obtained for the market samples

4. To evaluate the survival of *Listeria monocytogenes* in domestic cooking

5. To estimate the risk of ingesting *Listeria monocytogenes* through consumption of traditionally processed fish contaminated with the pathogen

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Ghana fishery sector

The fisheries sector in Ghana is an important player in the country's economy. It is estimated to have contributed about 3.9% of the nation's gross domestic product (GDP) and 11% of the Agricultural GDP in 2008 (Bank of Ghana, 2008). These GDP and AGDP figures stood at 3% and 5% respectively in 1997 (Sarpong, 2008), indicating the significant increases in the contributions of the sector to poverty reduction and provision of sustainable livelihoods over the years.

The sector is currently supported by fish supplies from the marine, freshwater and aquaculture fisheries. Marine fisheries are the major suppliers, contributing more than 80% of the total annual fish catch in the country. Freshwater fishes are obtained from the Volta Lake (major supplier), reservoirs, coastal lagoons, and aquaculture (Sarpong, 2008).

2.1.1 Economic importance of the fishery sector

The fisheries sector makes tremendous contributions to the economic development of the country through its role in ensuring food security, GDP and foreign exchange earnings, as well as provision of employment (and thus poverty reduction).

2.1.1.1 Food security

This refers to the situation in which all people in a given population have enough food to eat at all times to be in good health, and to have assurance that this situation will not change in the future (FAO, 2009). Schmidhuber and Tubiello (2007) identified

availability of food, access to food, stability of incomes and food production, and utilization of food as the four crucial factors that must be adequately addressed if the goal of food security for all is to be achieved. Those four elements have accordingly been described as the 'pillars' of food security.

The fishery sector contributes to food security in the country by ensuring availability of animal protein food. As already indicated, fish protein contributes as much as 60% of the total animal protein consumed in Ghana (Steiner-Asiedu *et al*., 1991; Plahar *et al*., 1991). The average per-capita consumption of fish in the country is estimated to be between 20 and 25 kg, about twice the world average of 13 kg. Fish makes up 22.4% of food expenditure in all households and 25.7% in poor households, confirming the significant part it constitutes in Ghanaian diets (Atta-Mills *et al*., 2004).

2.1.1.2 Foreign exchange

Exports of fishery products account for over 50% of the country's earnings from non-traditional exports (NTE), and are reported to be the second most important NTE after horticultural products (BoG, 2008). In 2006, about 60,000 metric tons of raw and processed fish were exported; earning over US$80 million for the country (BoG, 2008). The Institute of Statistical, Social and Economic Research of Ghana (ISSER) indicates that in just one year, the share of fish and seafood in non-traditional agricultural export products increased from 25% (in 2000) to 33 % (in 2001) (BoG, 2008).

2.1.1.3 Employment

It is estimated that a total of 500,000 fishermen, fish processors, traders and boat builders are employed in the fisheries sector in Ghana. These 'employees' are estimated to form about 10% of the population (Aquay, 1992; Atta-Mills, 2004). A canoe census conducted for the marine fisheries in 2001 placed the number of artisanal fishermen at 120,000 (Bannerman and Cowx, 2002).

Apart from the fishermen, processors and traders who generally dominate the sector at the landing sites and market centres, a large number of people also obtain livelihood support through their involvement at different stages of the fish distribution chain. For example, labourers who pack, store, load, unload and transport fresh and processed fisheries products on foot or by trolley for short distances earn some income in the process. Others include export processors, cannery workers, fishmeal manufacturers and their staff, and those engaged in the production of packaging materials for different types of fish products. There are also those who supply production and processing inputs and services such as boat builders, mechanics, timber and fuel wood providers, and food vendors at landing and processing sites (Overa, 2002).

The involvement of people in the fish post-harvest chain comes in the form of full-time employment, seasonal involvement, and occasional or opportunistic involvement. This affords people in coastal and lakeshore areas, and all others interested in the fish trade to diversify their livelihood strategies (Atta-Mills *et al*., 2004).

The fishery sector is also one of the few sectors of the economy where there is considerable gender equality in the workforce; men are involved in fishing while women are the key players in on-shore post-harvest activities, undertaking fish processing, storage and trade activities.

2.2 Traditional fish processing in Ghana

It has been estimated that more than 80% of fish landed in the country is traditionally processed (Nketsia-Tabiri and Sefa-Dedeh, 2000; Adu-Gyamfi, 2006). Traditional fish processing is thus an important economic activity in Ghana. It serves as a source of income to many and also provides the main form in which fish is consumed.

According to Sefa-Dedeh (1989; 1993), traditional fish processing is often characterized by all or some of the following:

i. Low capital cost

No huge financial inputs are required to start a business in traditional fish processing. The basic requirements are a smoker, firewood as fuel, and fish for smoked fish, salting vat, salt, and fish for salted fish, and fish and sunlight for dried fish. These can be acquired/accessed rather easily with little initial capital.

ii. Labour intensive

Since the processes are not mechanized, every unit operation requires manual inputs.

iii. Time consuming

This results from the lack of mechanization in the processing activities. For example, in the production of sundried fish, the duration of drying is controlled by the weather, and can thus be prolonged during rainy seasons. This has implications on the safety of the final products

iv. Simple and small scale operations

Indigenous processing activities do not require any sophisticated technologies. Fish are also processed in small quantities. In most instances, quantities sufficient for marketing in a day are produced. This could partly be as a result of a lack of good storage facilities.

v. Poor quality control

No objective methods are employed to monitor processing. The readiness of products for the market is determined by the subjective judgments of the processors.

vi. Unhygienic processing conditions

Basic rules of personal and environmental hygiene are not satisfactorily practiced during traditional fish processing, thereby compromising the safety of products.

vii. Home based

Many traditional fish processors operate from their homes. From smoking

ovens built in front of homes to compounds in front of homes used for drying, processing areas, materials and activities are not clearly separated from those for households.

2.2.1 Methods of traditional fish processing

The methods of traditional fish processing in Ghana are smoking, salting, drying, fermentation, and frying (Nketsia-Tabiri and Sefa-Dedeh, 2000; Neequaye-Tetteh *et al.*, 2002). Among these, smoking is practiced the most; it is estimated that more than 60% of the country's fish landings are preserved by smoking (Adu-Gyamfi, 2006). Historically, smoked fish has also been the most patronized of all traditionally processed fish in Ghana (Orraca-Tetteh and Nyanteng, 1978; Adu-Gyamfi, 2006). This high level of smoked fish processing and consumption is also true for other West African countries (UNDP, 2002).

2.2.1.1 Smoking

The Ghana Standards Board has defined smoked fish as fish which has been exposed to smoke with the intention of deferring spoilage. Traditional fish smoking preserves fish through the combined effects of the following:

i. cooking: at high temperatures, the fish are cooked, thereby denaturing native enzymes which could cause deterioration, and kills vegetative microorganisms that could cause spoilage
ii. drying: heat from the burning wood contributes to the drying of the fish
iii. preservation value of the smoke: compounds such as methanol and phenols in the smoke have bactericidal properties (Ihekoronye and Ngoddy, 1985).

Smoked fish are placed into two categories based on the processing temperature at which they are produced. These are cold-smoked and hot-smoked fish (Ihekoronye and Ngoddy, 1985). The processes are accordingly called cold-smoking and hot-smoking respectively.

In cold-smoking, the internal temperature of the fish usually does not exceed 35^{o}C. Generally, a range of 30-40^{o}C for 30-60 minutes is typical (Cofie, 2003). It is mostly practiced in technologically advanced countries. Cold-smoked fish are neither well dried nor cooked due to the low temperatures employed. Hence, they have high moisture contents and short shelf-life, typically 3 days (Cofie, 2003). They also require cooking before consumption.

In hot-smoking, the processing temperature is usually ≥90^{o}C. The internal temperature of fish typically exceeds 60^{o}C. The products have relatively low moisture content and thus have longer shelf life. Hot-smoked fish are cooked and can therefore be consumed without further heat treatment (Bannerman and Cowx, 2002).

Hot-smoking is the method employed in traditional fish smoking in Ghana, and in many developing countries (MOFA, 1999; UNDP, 2002). There are two forms of hot-smoking, namely wet hot-smoking and dry hot-smoking. They differ in their duration and the final moisture content of the products. Whilst wet hot-smoking generally takes 1-2 hours and yields products with moisture contents of 40-55%, dry hot-smoking takes 10-18 hours and yields a product with low moisture contents (10-15%) (UNDP, 2002).

2.2.1.2 Salting

In salting, fish is preserved by significantly reducing its moisture content through the osmotic effect of common salt (NaCl). The lowered water activity and residual salt in the resulting products discourage the growth of most microorganisms (Essuman, 1982). However, salt tolerant (also called halotolerant) microorganisms such as *Staphylococcus aureus* and *Listeria monocytogenes* have the potential to grow in such products.

There are two main types of salted fish products in Ghana, namely salted dried fish and salted fermented fish (Nketsia-Tabiri and Sefa-Dedeh, 2000). In salted dried fish, after extraction of water from fish using salt, the fish are further dried under the sun. A product made through this process is *koobi* (salted dried tilapia) and *kako* (salted dried ray fish). Products such as *momoni* are obtained by fermenting fish after the salting operation.

There are four methods of salting, namely brining, pickling, kench curing, and Gaspé curing (Horner, 1997). During brining, fish is immersed in a slightly saturated salt solution for a few minutes and removed. This usually serves as a preliminary step for other unit operations such as smoking and drying. The brining step is often done to impart a desirable flavour to the product (Horner, 1997). When fish are salted by immersion in saturated salt solution for long periods, the process is called pickling. This is often employed to preserve fatty fish, since the immersion prevents direct contact with atmospheric oxygen, thus preventing rancidity reactions.

In Kench curing, fish are cut open and arranged such that a layer of salt separates any two layers of fish (Horner, 1997). The exudate is drained off as waste. The method is employed in salting non-fatty fish and gives dry products. The only difference between Gaspé curing and Kench curing is that in the former, the exudate serves as a salt solution into which the fish are immersed for a further 2-3 days, after which they are removed and dried (Horner, 1997).

The exudate in both Kench and Gaspé curing is called 'pickle'. When water is extracted from fish so that the moisture content is reduced from 82% to about 54%, the fish is called green cured (Ihekoronye and Ngoddy, 1985).

2.2.1.3 Fermentation

In this method, fish is mixed with salt and made to ferment. The method preserves fish by increasing its acidity. Essuman (1992) defined fermented fish as any fishery product resulting from the enzymatic and/or microbial degradation of fish either in the presence or absence of salt. The products are usually used as condiments (Cofie, 2003).

2.2.1.4 Drying

The low humidity and ambient temperatures of tropical environments are made use of to dry fish, particularly bony fish such as tilapia. The final products usually have a moisture content of 14-30%. The fish are usually split asymmetrically longitudinally and arranged under the sun to dry (Essuman, 1992). The hygienic conditions of drying are generally unsatisfactory. Artisanal fish processors are known to dry their fish on the bare ground or on mats spread out on the bare ground.

2.3 Consumption of traditionally processed fish in Ghana

As was mentioned in Section 1.5 (Chapter One), Ghana records high per capita fish consumption. With a value of 20 – 25kg, the country's per capita fish consumption is nearly twice the world average of 13kg (BoG, 2008). In agreement with these figures, fish has been the preferred and cheapest source of animal protein in Ghana (Steiner-Asiedu *et al*., 1991; Adu-Gyamfi, 2006). About 75% of total annual fish landings are consumed locally (Sarpong, 2008, BoG, 2008). The high consumption rate is mainly due to high availability and low price of the commodity compared to other sources of animal protein.

Given that about 80% of fish landings in Ghana is traditionally processed (smoked, salted, fried, or dried), it can be argued that a greater quantity of the 75% of total annual fish landings consumed in the country is traditionally processed. By extension, it can be hypothesized that traditionally processed fish probably constitutes a greater portion of the 60% animal protein provided by fish in Ghanaian diets, and that a greater portion of the estimated 22.4% household expenditure on fish is made on the traditionally processed fish. Ghana can therefore be said to be a heavy consumer of traditionally processed fish.

The products are mostly purchased from informal markets in both urban and rural areas. These informal markets are an essential component of the informal sector in Ghana.

2.4 The informal food sector

The informal sector has been defined as employment or production that takes place in

small, unregulated and/or unregistered enterprises (Chant, 1999). Generally, the sector is characterized by reliance on indigenous resources, family ownership, small-scale operations and unregulated and competitive markets (Munhande and Makaye, 2008). It offers significant economic benefits globally and in Ghana, it is estimated to contribute as much as 58% GDP (ILO, 2002).

The informal sector has several 'sub-sectors', each involved in businesses related to a particular service, example barbering shops, unregistered tailors and seamstresses, and street hawkers. Those whose activities are related to food and its distribution form the informal food sector.

According to FAO (2007), the informal food sector (IFS) actors include small-scale producers and processors, manufacturing enterprises, traders and service providers who undertake either legal or unrecognized activities related to food. Market women selling livestock and horticultural produce, caterers, street food vendors (including those at fixed kiosks, mobile stands, those who sell from vehicles such as carts and bicycles trucks) are all players in IFS (FAO, 2007). In Ghana, small-scale farmers, persons in markets selling various kinds of fresh, traditionally processed and industrially processed food items, and ready-to-eat street food vendors are included.

IFS offers convenience in food purchasing at a low cost to consumers, especially those in the lower and middle classes, the urban poor, office workers and tourists, while providing income to those who sell the foods. It also contributes to making food available in marginal urban districts that are distant from major city commercial centres (FAO, 2007) and thus contributes to food security.

The indicated benefits notwithstanding, there are some challenges associated with IFS. Argenti (2000) argued that as a result of the generally unregulated status and lack of formal legal support, the informal food sector lacks the appropriate inputs for improvements in food hygiene. Consumers therefore face food safety risks in purchasing foods from the sector. This is true in Ghana, (as it is in many other developing countries) where the safety of foods sourced from IFS cannot be guaranteed due to poor hygiene control and monitoring. For example, Mensah *et al*., (2002) isolated enteroaggregative *Escherichia coli*, *Salmonella arizona*, and *Shigella sonnei* in some street foods in Accra.This notwithstanding, many are of the view that the sector should not necessarily be equated to poor quality food (FAO, 2007).

2.5 Safety of traditionally processed fish on informal markets

As a result of the generally unhygienic conditions under which fish is traditionally processed and sold, the main safety consideration is the microbiological quality of the products. Adu-Gyamfi (2006) conducted a study on the microbiological quality of smoked fish in some informal markets in Accra and found that the safety of the products was compromised. Microorganisms isolated included *Escherichia coli, Klebsiella pneumonia,* and *Proteus mirabilis.* Nyamekye (2000) also isolated *Staphylococcus aureus, Escherichia coli, Micrococcus sp* and *Proteus sp* from smoked herrings and mackerel purchased from informal markets in Accra. Oppey (2002) found *Aspergillus sp*, *Penicillium sp*, and coliforms in some smoked fish products from informal markets in Accra.

In addition to microbial contamination resulting from improper handling practices, processing conditions could also mar the chemical safety of processed fish prior to sales on informal markets. For example, in smoked fish, the deposition of carcinogenic compounds in wood smoke could make the products unsafe. These compounds include polycyclic aromatic compounds (e.g. 3,4-benzopyrene and pyrene) and nitrosamines (Rahman and Perera, 2007).

In salted fish, the key concerns are the chemical and microbial quality of the salt used. Additionally, high levels of magnesium and calcium in salt result in bitter and toughened products (Collignan and Raoult-Wack, 1994).

In dried fish products, the key concern is possible microbial contamination arising from the practice of drying fish on the bare ground or very close to the same. For fermented fish products, the generally uncontrolled fermentation could result in the growth of potentially dangerous microorganisms in the products (Guizani and Mothershaw, 2007).

2.6 Bacterial pathogens associated with fish

Fish has associated bacteria pathogens that could be present in the skin, gills or gut. These organisms have been categorized into two groups: indigenous and non-indigenous pathogenic bacteria (Nickelson and Finne 1992; Huss *et al*. 1995).

The indigenous pathogenic bacteria are commonly found in the aquatic environment; they are present on the live fish and their presence in the final product is probable. They include *Listeria monocytogenes, Clostridium botulinum*, *Aeromonas hydrophila*

and *Vibrio* sp (Huss *et al*. 1995, Nickelson and Finne 1992). Non-indigenous pathogenic bacteria are normally associated with human or warm-blood animals and their faeces, and are not naturally present in fish. They are therefore contaminants. *Salmonella* sp, *Escherichia coli* and *Staphylococcus aureus* are among such pathogens (Huss *et al*. 1995; Nickelson and Finne 1992).

Matte *et al.*, (1994) also categorized the pathogenic bacteria associated with fish (and sea food in general) into three groups as follows:

a. bacteria which are normal components of the marine or estuarine environment: *Vibrio parahaemolyticus, Vibrio cholera, Vibrio vulnificus, Clostridium botulinum, Aeromonas hydrophilia,* and *Listeria monocytogenes*
b. enteric bacteria whose presence is attributed to faecal contamination: *Escherichia coli, Shigella sp, Campylobacter sp,* and *Yersinia enterocolitica*
c. bacteria that contaminate fish during processing: *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus,* and *Clostridium perfringens*

These microorganisms, when present in fish and fish products and not eliminated before consumption, could cause various specific forms of food-borne diseases. Some pathogenic microorganisms implicated in food-borne illnesses arising from fish consumption are listed in Table 2.1.

Table 2.1: Some bacterial pathogens implicated in food-borne disease outbreaks caused by fish

Pathogen	Associated fish
Listeria monocytogenes	Cold-smoked and hot-smoked fish, salted fish
Salmonella sp	Tilapia, carp, prawns, catfish
Shigella sp	Shellfish
Yersinia enterocolitica	Aquaculture fish
Vibrio cholera	Prawns, squid, shellfish
Vibrio parahaemolyticus	Most finfish, shellfish,
Vibrio vulnificus	Finfish, mussels, prawns, oysters
Campylobacter jejuni	Shellfish

Source: Fehlduson (1999)

It is seen that the classification systems by Huss *et al*. (1995), Nickelson and Finne (1992), and Matte *et al.*, (1994) all recognize *Listeria monocytogenes* as a bacterial pathogen associated with fish.

2.7 *Listeria monocytogenes*

L. monocytogenes is one of six species of bacteria in the genus *Listeria.* The other species are *L. ivanovii, L. seeligeri, L. grayi, L. welshimeri,* and *L. innocua* (Prentice and Neaves, 1992). The genus belongs to the *Clostridium* sub branch with *Staphylococcus, Streptococcus, Lactobacillus,* and *Brochothrix* (Montville and Mathews, 2005). The *Listeria* species are identified by a few biochemical traits. These include tests for acid production from D-xylose, L-rhamnose, methyl-D-mannoside, and D-mannitol (Table 2.2). The ability to lyse red blood cells differentiates *L. monocytogenes* from the non-pathogenic strains (Jay *et al.*, 2005).

Table 2.2: Differentiating characteristics of *Listeria* species

		Acid production from				
Species	**β-haemolysis***	**Glucose**	**α-Methyl-D-mannoside**	**Rhamnose**	**Xylose**	**Mannitol**
monocytogenes	+	+	+	+	-	-
Innocua	-	+	+	v	-	-
Ivanovii	++	+	-	-	+	-
Seeligeri	(+)[1]	+	v	-	+	-
Welshimeri	-	+	+	v	+	-
Grayi	-	+	+	-	-	+

*****Horse or sheep blood [1]Washed sheep blood (+)[1] Weak reaction v: different strains give different reactions *Source*: Prentice and Neaves (1992)

Within the genus *Listeria,* only *L. monocytogenes* and *L. ivanovii* are pathogenic; the former, mainly to humans and the latter, to animals, particularly sheep (Prentice and Neaves, 1992).

L. monocytogenes is Gram-positive, facultatively anaerobic, non-sporing, rod-shaped motile bacterium. Other cellular shapes, such as palisade, cocci and Y forms have also been observed (Garbutt, 1997). Apparently, the shape occurring at any time depends on the culturing conditions (Garbutt, 1997). It shows a unique tumbling motility at 20-25^{o}C but not at 35^{o}C (Prentice and Neaves, 1992). The organism is psychrotrophic and grows over a temperature range of 0° to 45°C, with an optimum around 37°C (Jay *et al*, 2005). It grows slowly at colder temperatures and is generally known to be killed at temperatures >50^{o}C (Black, 1999).

L. monocytogenes has been found to grow at water activities (a_w) ≥0.92 with sodium chloride (NaCl) as the solute. Generally, however, the organism grows best at a_w ≥0.97 (Jay *et al.,* 2005). While the minimum a_w for growth for most strains is 0.93,

some are able to grow at a_w 0.90. The organism is able to survive for long periods at a_w as low as 0.83 (Montville and Matthews, 2005). Accordingly, *L. monocytogenes* is recognized as the second food-borne pathogen (after the staphylococci) with the ability to grow at a_w values <0.93 (Cofie, 2003).

When foods containing *L. monocytogenes* are heated, the thermal resistance of the organism increases as the water activity of the foods decrease. In one study, when Scott A strain of *L. monocytogenes* in liquid whole egg was heated at 60°C for 3.5 minutes, the *D* value (decimal reduction time, which is the time required to reduce the numbers of microorganisms or their spores by 90% at a specified temperature (Atlas, 1997) was 2.1 minutes. However, the same strain in liquid whole egg to which 10% NaCl was added and heated at 63°C for 3.5 minutes had a *D* value of 13.7 minutes (Bartlett and Hawke, 1995). It is seen that although a higher temperature (63°C) was used for liquid whole egg with 10% salt, the *D* value was about four times that of the liquid whole egg without salt. This was because the 10% NaCl lowered the a_w of the product from 0.98 to 0.915 (Linton *et al*., 1990).

L. monocytogenes also exhibits remarkable tolerance to high salt concentrations. It grows to high levels at concentrations of 6.5%, grows considerably at 10-12%, and survives for long periods at higher concentrations of NaCl. In salty food systems, as temperature is lowered, the ability of the pathogen to survive at high salt concentrations increases (Ryser and Marth, 1988). This has implications on the safety of cured products such as salted fish.

L. monocytogenes can grow at pH levels between 4.4 and 9.4 in laboratory media. Below pH 4.3, the cells survive, but may not grow. It is reported that organic acids such as acetic, citric and lactic acid at 0.1% concentration inhibit the growth of the organism (Montville and Matthews, 2005).

2.7.1 Heat resistance of *Listeria monocytogenes*

Several studies have been conducted on the effect of heat on the survival and growth of *L. monocytogenes* in several food items. The decimal reduction time (*D* value) and z values for the organism in some foods are presented in Table 2.3.

Table 2.3: Selected findings on the thermal destruction of *L. Monocytogenes*

Strains tested/state	Number of Cells (ml)	Heating Menstrum	Heating Temp. (^oC)	D value (sec)	z value (^oC)	Reference
Scott A, free suspension	~10^5	Sterile skim milk	71.7	1.7	6.5	Bradshaw *et al*, 1987
	~10^5	Sterile skim milk	71.7	2.0	6.5	Ryser and Marth, 1988
	~10^5	Sterile skim milk	71.7	0.9	6.3	Bradshaw *et al.*, 1985
Scott A, intracellular	~10^5	Whole raw milk	71.7	1.9	6.0	Brunning *et al.*, 1986
Scott A, free suspension	~10^5	Whole raw milk	71.7	1.6	6.1	Brunning *et al.*, 1986
F5069, intracellular	~10^6	Sterile whole milk	71.7	5.0	8.0	Bruning *et al.*, 1986
F5069, free suspension	~10^6	Sterile whole milk	71.7	3.1	7.3	Brunning *et al.*, 1986
Scott A, free suspension	~10^5	Ice cream mix	79.4	2.6	7.0	Bradshaw *et al.*, 1987
	~10^8	pH 5.9, meat slurry	70.0	13.8	NR	Boyles *et al.*, 1990
	~10^7	Liquid whole egg	72.0	36.0	7.1	Boegeding *et al.*, 1990
Ten strains	~10^7	Irradiated ground meats	62.0	61.0	4.92	Farber, 1989
Chicken/meat isolate	~10^5	Beef	70.0	NR	7.2	Mackey *et al.*, 1990
	~10^5	Minced chicken	70.0	NR	6.7	Mackety *et al.*, 1990

Source: (Jay *et al*., 2005).

D value (decimal reduction time): Time taken to reduce the number of microorganism or spores in a sample by 90% at a specified temperature. **Z value**: Tempreture increase required to reduce the D value to 10% of its original value (Prescott *et al.*, 1995). **NR**: Not Reported.

For dairy products, the reported *D* values suggest that high-temperature short-time (HTST) treatment (71.7◦C for 15 seconds) is adequate to reduce normally existing numbers of the pathogen to below detectable levels. Farber *et al*., (1998) subjected milk that was naturally contaminated with *L. monocytogenes* at 104 cells/ml to HTST treatment and found no viable cells at processing temperatures of 69°C or above. Jay *et al.,* (2005) have however indicated that the low-temperature, long-time (LTLT) heat treatment method (62.8°C for 30 minutes) has a greater lethal effect on the pathogen. Mackey and Bratchell (1989) also concluded that the margin of safety is greater for the LTLT treatment than the HTST treatment. For non-dairy products, the thermal resistance of *L. monocytogenes* varies with the type and composition of the foods.

The biological structure and composition of foods has implications on the thermal resistance of *L. monocytogenes*; the organism has greater heat resistance in foods with high fat content owing to the heat-shielding effect of the fat. Embarek (1994) studied the heat resistance of two strains of *L. monocytogenes* in cooked cod and salmon fillets and found that both strains were about four times more heat resistant in salmon than in cod, as a result of the relatively more fatty nature of salmon. *D* values for liquid whole egg and meat products are also reported to be generally higher than for milk (Jay *et al*., 2005). The higher protein and fat content in the whole eggs and meat products than in the milk account for the difference.

Farber (1989) found that cure ingredients increased the *D* value for the pathogen. The author found the *D* value for sausage-type meat at 62°C to be 61 seconds (Table 2.4).

The *D* value however increased to 426 seconds (7.1 minutes) when cure ingredients were added. This suggests that the components of the cure ingredient (nitrite, dextrose, lactose, corn syrup, and 3% (w/v) NaCl) offered some thermal protection to the organism. Mackey *et al*., (1990) also found that when 30% fat, 3.5% NaCl, 200 ppm nitrite, and 300 ppm were added to ground beef, the *D* value approximately doubled.

Conflicting results have been obtained for the effect of initial sublethal heating of *L. monocytogenes* on the resistance of the organism to subsequent heat treatments. While some studies observed no effect (Bradshaw *et al.*, 1985; Bunning *et al.*, 1990), others have reported increased resistance (Fedio and Jackson, 1989; Farber and Brown, 1990; Linton *et al.*, 1990;). Linton *et* al., (1990) found that when some strains were heat shocked at 48^{o}C for 20 minutes, their heat resistance increased at 55^{o}C. Fedio and Jackson (1989) reported an increased resistance at 60^{o}C after the organisms were heat-shocked at 48^{o}C for 60 minutes. Farber and Brown (1990) also found that when 10 strains at a concentration of 107 cells/g in a sausage mix were heat shocked at 48°C for 30 or 60 minutes, no significant increase in heat resistance was observed after subsequent exposure to 62^{o} or 64^{o}C. However, in the same study, those shocked for 120 minutes recorded increased resistance at 64^{o}C. The cells also maintained their heat resistance for at least 24 hours when stored at 4^{o}C.

2.7.2 Occurrence of *L. monocytogenes*

The pathogen is highly ubiquitous (Prescott *et al*., 1995; Garbut 1997; Black, 1999; Jay 2003). It occurs in soil, dust, fresh and salt water, decaying vegetation, silage, slaughter house waste, sewage effluent (Garbutt, 1997; Jay *et al*., 2005), and as

biofilms on food processing equipments (Mauro *et al.*, 2008) and hospital equipments. Jemmi and Keusch (1994) isolated the organism in fish from Swiss freshwater fish farms.

L. monocytogenes has the ability to colonize and adapt to various environments as a result of its unique tolerance to a wide range of temperatures, pH and salt concentration (Mauro *et al*.,2008; Jay *et al*., 2005), and also because of its ability to form biofilms (Jay *et al.*, 2005).

2.7.3 Foods frequently contaminated with *L. monocytogenes*

As a result of its widespread distribution in the environment and hardy nature, *L. monocytogenes* contaminates a wide range of foods. In fact, some international food regulators have opined that it is impossible to produce foods that are practically free of the organism (Montville and Matthews, 2005). The organism has been found in raw milk and dairy products, fresh and frozen meat and meat products, poultry products, seafood, and on fruits and vegetable products (Jay *et al*., 2005). Some specific foods in which the pathogen has been isolated include whole milk, skim milk, soft cheese, processed meats, red meat, vacuum packaged beef and poultry products, lettuce, cold-smoked and hot-smoked fish, salted fish, coleslaw, and fried rice (Montville and Matthews, 2005; Jay *et al.*, 2005). These foods can therefore serve as suitable vehicles of transmission for the organism, particularly when they are processed, stored or prepared under conditions that facilitate contamination and encourage the growth of the pathogen.

2.7.4 Persistence of *L. monocytogenes* in foods

As a result of its hardy nature (growth over wide range of temperature (0-45^{o}C) and pH (4.1 – 9.6), in high salt concentration and at low water activity), *L. monocytogenes* is able to survive for long periods in foods. At levels of 104–105/g, the pathogen survived in cottage cheese for up to 28 days when held at 3^{o}C (Ryser and Marth, 1988). It also survived for 130 days in cold-pack cheese stored at 4^{o}C in 0.30% sorbic acid (Ryser and Marth, 1988**)**. Shelef (1989**)** also found that the count of *L. monocytogenes* remained unchanged in ground beef and liver for over 30 days, although the standard plate counts (SPC) increased during that same period.

Glass and Doyle (1990) challenged eight processed meats with five strains of *L. monocytogenes* under 12 weeks storage at 4.4^{o}C. The pathogen not only survived on all products, but increased in numbers by 3 to 4 logs in most. The products with high initial pH (such as poultry products) recorded the highest growths.

2.7.5 Transmission of *L. monocytogenes* to humans

L. monocytogenes is widely distributed and therefore has many potential routes to infect humans (Fig. 2.1 and 2.2). The modes of transmission include vertical (mother to child), zoonotic (contact with animal to man), and nosocomial (hospital acquired) (FAO/WHO, 2004).

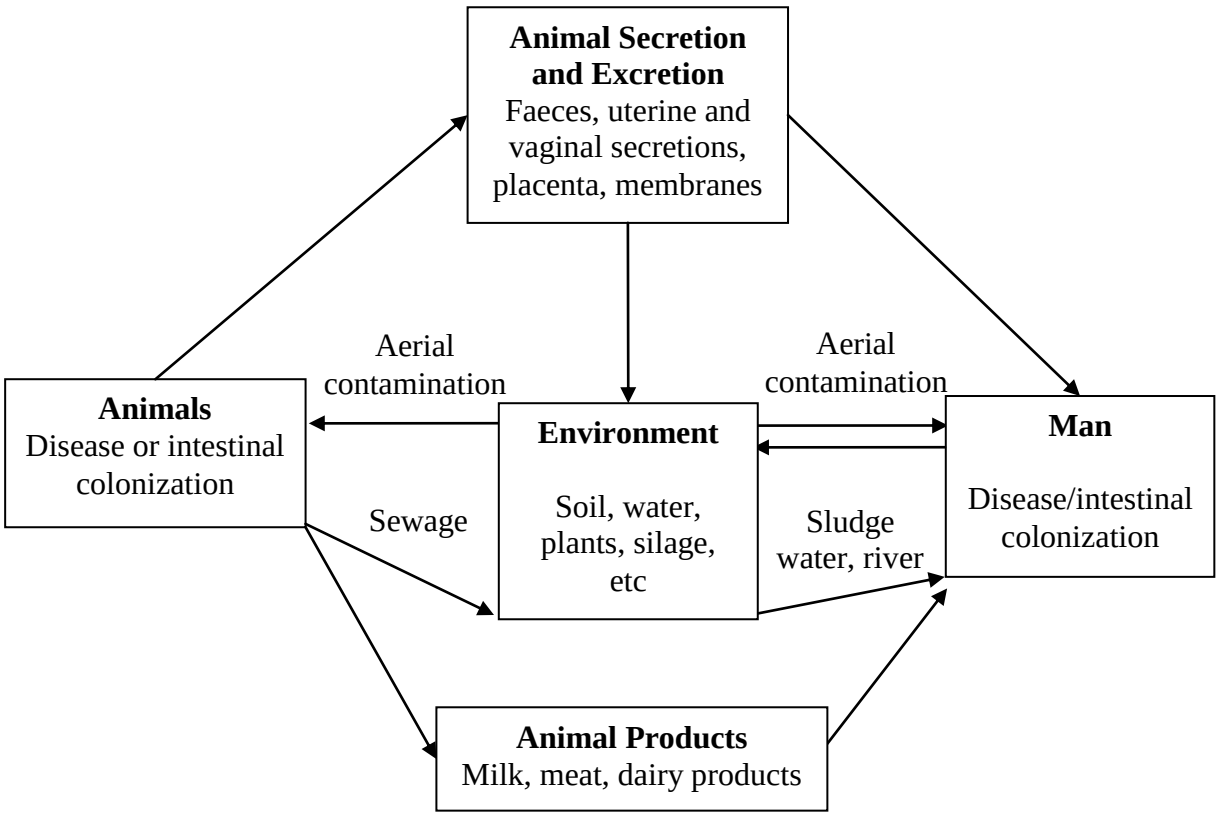

Fig. 2.1: Ways by which *L. monocytogenes* is distributed in the environment
Source: Jay *et al.*, (2005)

Fig. 2.1 shows that humans get infected with *L. monocytogenes* either through the environment or through food. Humans, once infected, also contribute to the spread of the organism in the environment.

Fig. 2.2 focuses on food as the principal means of infection, and also shows how humans have the potential of releasing the pathogen into the environment, with the result that foods ultimately get contaminated and deliver the organism back to humans.

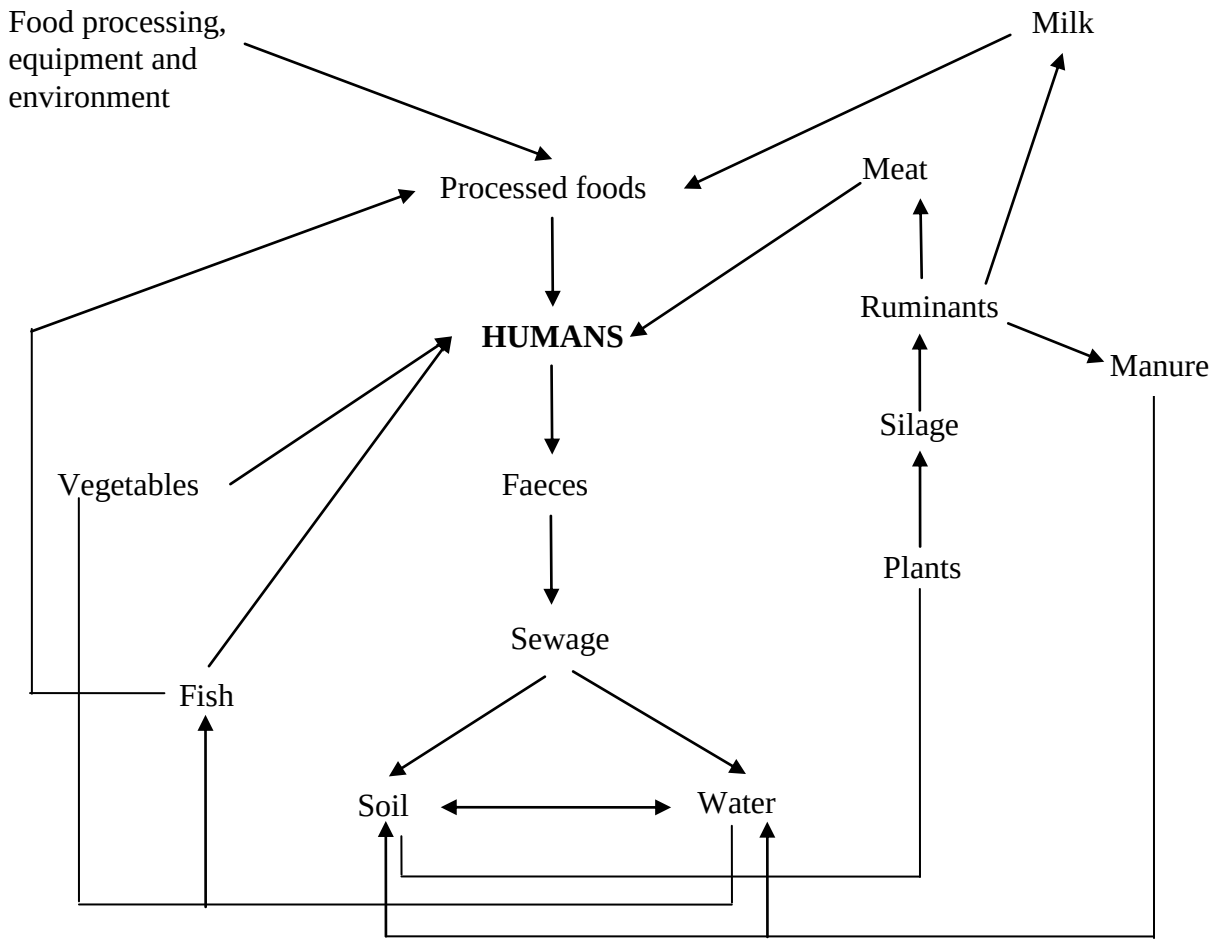

Fig. 2.2: Potential routes of transmission of *L. monocytogenes* to humans
Source: Montville and Matthews (2005)

It is generally recognized that most cases of human infections involve food-borne transmission, as is apparent in Fig. 2.2. However, this has not always been the accepted knowledge.

When the first case of food-borne infection occurred in 1953 in which the stillbirths of twins was linked to consumption by the mother of raw milk from a cow with listerial mastitis (FAO/WHO, 2004), the significance of foods as a mode of transmission was not given much consideration until the 1980s when several large outbreaks of

listeriosis linked to common foods occurred in North America and Europe. It was then that the significance of foods as the primary route of transmission for human exposure to *L. monocytogenes* was recognized (Broome *et al.*, 1990; Bille, 1990). Following this, there has been a strong interest in the control of the pathogen in foods (FAO, 2000). Today, contaminated food is estimated to be responsible for about 90% of food-borne illnesses caused by *L. monocytogenes.*

2.7.6 The disease caused by *Listeria monocytogenes*

Listeria monocytogenes causes a fatal food infection called listeriosis. According to the United States Centre for Disease Control and Prevention (CDC, 2009), listeriosis is clinically defined when the organism is isolated from blood, cerebrospinal fluid, or an otherwise normally sterile site in the body, such as the placenta and foetus. It is therefore only positively diagnosed by culturing the organism from blood, cerebrospinal fluid, or stool.

Once infection occurs, the organism is able to enter and multiply in the host's monocytes, macrophages, or polymorphonuclear leukocytes. This offers it access to the brain and the placenta (Montville and Matthews, 2005). CDC (2009) indicates that the pathogenicity of *L. monocytogenes* rests on its ability to cause systemic infection (survival and multiplication in body cells).

The disease occurs rarely but has very high fatality rates, usually 20 – 30% (Kalliopi *et al.*, 2008). The mortality from meningitis, septicaemia and perinatal/neonatal infection caused by *L. monocytogenes* are respectively estimated at 70%, 50% and 80% (CDC, 2009). Persons at high risk include pregnant women, the very young (less

than 1 year), the very old, and the immunocompromised (Kathariou, 2002). A non-invasive form of the disease characterized by febrile gastroenteritis suggests that persons with no predisposing conditions may be affected (Lorber, 2007).

It has been found that some healthy individuals are asymptomatic faecal carriers of the pathogen. These include pregnant women, patients with gastroenteritis, slaughterhouse workers, laboratory workers handling *Listeria,* food handlers, patients undergoing haemodialysis, and some healthy people (Montville and Matthews, 2005). It is estimated that about 1-10% of humans may be intestinal carries of the pathogen (Montville and Matthews, 2005), and that faecal carriers amplify outbreaks through secondary transmission (i.e. transfer of the organism from the faeces of the first victim to another person) (Montville and Matthews, 2005). Interestingly, some pregnant women who are asymptomatic carriers are able to have normal pregnancy outcomes (Montville and Matthews, 2005).

The first human case of listeriosis was reported in 1929 (Jay *et al.*, 2005). Sporadic incidents have followed since then, with high fatalities per occurrence. It is reported that between 1986 and 1988, human listeriosis increased in England and Wales by 150% (Jay *et al.,* 2005). Between 1983–1987, 775 cases were reported in Britain, with 219 (28%) deaths (Jay *et al.,* 2005).

Data prospectively collected by the United States Centre for Disease Prevention and Control (CDC, 2009) suggests that at least 1600 cases of listeriosis occur annually in the USA, with 415 deaths per year. Table 2.4 lists some incidents of listeriosis and their attendant fatalities.

Table 2.4: Some food-borne listeriosis outbreaks

Year	Source	Cases/Deaths	Location
1953	Raw milk	2/1	Germany
1959	Fresh meat/poultry*	4/2	Sweden
1960-1961	Various/unknown	81/?	Germany
1966	Milk/products	279/109	Germany
1979	Vegetables/milk**	23/3	Boston
1980	Shellfish	22/6	New Zealand
1981	Cole slaw	41/18	Canada
1983	Pasteurized milk**	49/14	Boston
1983-1987	Vacherin Mont D'Or	122/34	Switzerland
1985	Mexican-style cheese	142/48	California
1986-1987	Vegetables**	36/16	Philadelphia
1987-1989	Pate	366/63	United Kingdom
1987	Soft cheese	1	United Kingdom
1988	Goat milk cheese	1	United Kingdom
1988	Cooked, chilled-chicken	1	United Kingdom
1988	Cooked, chilled-chicken	2	United Kingdom
1988	Turkey franks	1	Oklahoma
1989	Pork sausage	1	Italy
1988	Alfalfa tablets	1	Canada
1989	Salted mushrooms	1	Finland
1989	Shrimp	9/1	United States
1989	Pork sausage	1	Italy
1990	Raw milk	1	Vermont
1990	Pork sausage	1	Italy

*Suspected **Epidemiologically linked; organism not found

Table 2.4 continued

1990	Pate	11/6	Australia
1991	Smoked mussels	3/0	Australia
1992	Smoked mussels	4/2	New Zealand
1992	Goat meat (California)	1	Canada
1992	Port tongue in jelly	279/85	France
1993	Pork rillettes	39/0	France
1994	Chocolate milk	52/0	USA
1994	Pickled olives	1	Italy
1995	Brie cheese	17/0	France
1998-1999	Wieners	101/21	United States
1998	Butter	25/24	Finland
1999-2000	Pork tongue in jelly	26/7	France
2000	Pork	32/31	France
2000-2001	Homemade Mexican-style cheese	12/0	United States
2002	Deli turkey meat	46/7	10 USA States
2009	Red meat	53/20	Canada

*Suspected
**Epidemiologically linked; organism not found
Source: CDC (2009); Jay *et al*.,(2005)

2.7.6.1 Foods implicated in listeriosis outbreaks

Ready-to-eat (RTE) foods are mostly implicated in listeriosis outbreaks (Mauro *et al*., 2008). This is because those foods are often not given any further heat treatment before consumption. Among the RTE foods, meat and poultry products are reported to be the most frequently implicated vehicles of transmission (Jay *et al.*, 2005). Other foods implicated in listeriosis outbreaks include red meat (2009, Canada), pork (2000, France), and butter (1998, Finland). Cooked chilled foods, vacuum-packed meat and fish products, and smoked fish (Oroczo, 2000) are also known vehicles for the pathogen (Table 2.5).

2.7.7 Occurrence of *Listeria monocytogenes* in fish

Several studies have reported the incidences of *Listeria* spp. and *L. monocytogenes* in fish products such as cold-smoked fish, hot-smoked fish, and salted fish (Oroczo, 2000; Heinitz *et al*., 2000; Rørvick *et al*. 1995). Lindqvist and Westoo (2000) found the organism in smoked trout in Sweden. Kwiatek (2000), in a study on the occurrence of *L. monocytogenes* in foods of animal origin, found the pathogen in raw and smoked fish in Poland. Salihu *et al*. (2008) detected the pathogen in smoked fish in Sokoto, Nigeria. Conflicting results have been reported on the occurrence of *L. monocytogenes* in marinated fish; some studies have found the organism in the product, whiles others reported its absence (Kwiatek, 2000).

Ikeh *et al*., (2010), in a study to identify the incidence and pathogenicity profile of *Listeria sp* from food and environmental samples in Nigeria, isolated the organism from both fresh and dried fish. Relative to the other foods found to contain the organism, the fish samples had a low incidence of 40% (beef 80%, poultry 70%, and vegetables 85%). They attributed the low incidence in fish to a possible low level of contamination of the water bodies from which the fish were caught.

Prior to recent research, such as those conducted by Salihu *et al*., (2008) and Ikeh *et al*., (2010), some studies reported the absence of *Listeria* sp in tropical fish on the basis of unsuitable environmental conditions (Kamat and Nair, 1994; Karunasagar *et al*., 1992; Manoj *et al*., 1991). After detecting *L. monocytogenes* in 17.2% of finfish and 12.1% of shellfish in India, Jeyasekaram and Karunasagar (1996) attributed the perceived absence of the pathogen in tropical fish to inadequate isolation procedures.

2.7.7.1 Factors influencing initial load of *Listeria monocytogenes* in fish

The initial load of *Listeria* species in fish or seafood is influenced by such factors as origin (wild or farmed), season, fishing technique, handling and storage conditions. For example, a greater incidence of *Listeria* species including *L. monocytogenes* has been reported in fresh water fish (81%) than in marine fish (30%) (Colburn *et al*. 1990). Beumer (1997) indicated that once present in fish (fresh or processed), the ability of *L. monocytogenes* to grow depends on those same factors that generally affect the growth of microorganisms in food, namely intrinsic factors (pH, water activity, preservatives in the food), extrinsic factors (storage temperature, atmosphere in the package) and implicit factors (competition with other microorganisms).

2.8 Regulation of *Listeria monocytogenes* in foods

The regulation of *L. monocytogenes* in foods is a controversial issue under a continued international debate that is anticipated to go on for years. While most countries in the European Union (EU) have set **tolerance levels** (allowable limits of counts) for the pathogen, the United Kingdom and the United States of America have declared a **zero-tolerance** for the pathogen (Gallagher *et al*., 2003).

The EU countries argue that since *L. monocytogenes* is so widespread, it is practically impossible to produce foods that are free of the pathogen. The tolerance levels set by these countries therefore define acceptability and unacceptability of foods based on the counts of the organism in the foods. In some of the EU countries, food products that historically have caused human listeriosis are placed in a special category and are monitored more strictly than those that have never been implicated in the disease.

Generally, the tolerance level policy asserts that foods should be *L. monocytogenes* free **if possible**, or have the lowest counts possible (Montville and Matthews, 2005). Specifically, foods for high-risk groups (pregnant women, the very young, the elderly, and the immunocompromised) must be *Listeria*-free. All other foods may contain up to 100CFU/g. In January 2006, Commission Regulation (EC) No. 2073/2005 was implemented in the EU (Kalliopi *et al*., 2008). This policy defines a tolerance level of 100 CFU/g or ml for ready-to-eat products, and absence in 25g or ml for high-risk individuals.

The UK and the USA, which insist on a complete absence of the pathogen in 25g of all foods (zero tolerance policy) argue that since the infectious dose of the pathogen is not known and may vary for different people based on, for example, age and morbidity status, it is not sound to define acceptable levels of the pathogen in foods. They maintain that the infectious dose must be known before any limits can be defined. In the USA, as a result of the zero-tolerance policy, *L. monocytogenes* is considered an adulterant. Therefore, any food that contains the organism can be considered adulterated and could be seized or recalled (Jay *et al*., 2005).

Two arguments have been raised against the zero-tolerance policy. The first is that the incidence of listeriosis in the USA (~0.7 per 100,000 people) is the same as it is in the EU which allows <100CFU/g (Montville and Matthews, 2005). It appears, therefore, that the zero-tolerance offers no additional protection for consumers. Secondly, microbial specifications must be necessarily harmonized for the advancement of international trade. Therefore, under the zero-tolerance policy, foods that meet the

European Union's <100CFU/g tolerance would be rejected in the USA and the UK. Consequently, these countries could be charged with inhibition of free trade.

Another flaw in the zero tolerance policy is that current methods for detecting *L. monocytogenes* in foods have a 10 to 15% false negative rate (i.e. if 100 food samples **containing** *L. monocytogenes* were tested, the pathogen would **not** be detected in 10 to 15 of the samples) (Montville and Matthews, 2005). Questions have therefore been asked if it is sound to demand zero-tolerance when there is no 100% reliable test for detecting the organism in foods.

On country-specific regulations, Great Britain established four quality categories for ready-to-eat foods as follows (Gilbert, 1992):

Level in 25g	**Remarks**
Not detected	Satisfactory
<102 cells	Fairly satisfactory
102-103 cells	Unsatisfactory
>103 cells	Unacceptable

Lammerding and Farber (1994**)** reported that a 1993 Canadian *L. monocytogenes* compliance criteria placed ready-to-eat foods into three groups: category 1 included products linked to outbreaks, category 2 included those that had a self-life >10 days, and category 3 included those that either supported growth with a self-life ≤10 days or those that did not support growth.

Germany is strongly against the zero-tolerance policy. It asserts that the demands of the policy are unrealistic (Jay *et al*., 2005). However, the country indicates that products that contain >104 of the pathogen per gram of food must be subjected to automatic recall.

In France, foods for high-risk individuals must not contain *L. monocytogenes* in 25g samples. As generally argued by the EU, the French also assert that it is unrealistic to expect zero counts of *L. monocytogenes* in raw foods, particularly given the inevitable presence of the pathogen in food processing environments (Tompkin, 2002).

The International Commission on Microbiological Specification for Foods (ICMSF, 1996) has concluded that if the counts of *L. monocytogenes* do not exceed 100CFU/g of food at the point of consumption, the food is considered acceptable for individuals who are not at risk.

2.9 Detection *of L. monocytogenes* in foods

Generally, the detection of *L. monocytogenes* in foods involves enrichment, culturing on selective media, description of colonial characteristics on solid selective media, observation of cellular morphology, and finally biochemical and confirmatory tests (Prentice and Neaves, 1992). The enrichment step is considered particularly important as the organism is a poor competitor and would therefore not grow well in the presence of other microorganisms.

Among the protocols developed for the detection and isolation of *L. monocytogenes* are the ISO 11290 method and those by the United States Department of Agriculture

(USDA), US Food and Drugs Administration (FDA), and the United Kingdom Health Protection Agency (HPA). Whereas the ISO, USDA, and FDA protocols require pre-enrichment (primary and secondary) of samples in either University of Vermont broth (UVM) or Fraser Broth (FDA) for both detection and enumeration, the HPA method allows pre-enrichment for detection only, and not for enumeration (HPA, 2009).

Specifically, for detection and isolation, the USDA protocol requires primary enrichment in UVM broth, secondary enrichment in Fraser Broth, and plating on a selective medium. Colonies typical of *L. monocytogenes* are then taken through confirmatory identification procedures such as general biochemical tests, the Christie, Atkins, Munch-Peterson (CAMP) test, and genetic identification tests. The ISO and FDA protocols are similar in content.

2.10 Risk assessment

In the simple terms, risk assessment is an evaluation of the possibility of an undesirable event occurring. The exercise is premised on the prior assumption that the possibility exists for exposure to an injurious event or substance. Technically, the injurious substance is called 'a hazard', and the likelihood that it would occur to cause the undesirable outcome is referred to as 'risk'.

According to the Codex Alimentarius Commission (CAC, 2003), a hazard is any biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect. The Commission also defines a risk as a function of the probability of an adverse health effect and the severity of that effect, consequential

to a hazard(s) in food. Therefore, risk assessment evaluates the likelihood that exposure to a hazard would result in adverse health effects.

Risk assessment involves a systematic determination of the ability of a substance or organism to cause an adverse health effect (*hazard identification*)**,** evaluation of the probability of consumption of the hazard and the quantities likely to be ingested through food (*exposure assessment*)**,** description [in quantitative or qualitative terms] of the severity of the effect of the hazard following consumption (*hazard characterization*), and *risk characterization*, which is essentially a combination of the information gathered in the previous steps to fully explain the nature of the risk (Lammerding *et al.,* 2001; CAC, 2003). The outcomes of risk assessments are used in risk management (establishment and implementation of appropriate control measures) and risk communication (exchange of information among stakeholders of the risk assessment process) (CAC, 2003).

With regard to food safety, the commonest types of risk assessments are chemical and microbial risk assessment, which respectively focus on chemicals and pathogenic microorganisms as the hazards of concern. Regardless of the type, however, there are generally two forms of risk assessment, namely quantitative risk assessment and qualitative risk assessment. Whereas quantitative risk assessment offers numerical estimates of the risk (e.g. 1 in 100 chances of illness), qualitative risk assessments describe the nature of the risk (i.e. high, moderate, or low risk).

2.10.1 Microbial risk assessment

The concept of microbial risk assessment (MRA) rests on the determination of the

likelihood of consumers falling ill after intake of a food containing a food-borne microbiological hazard. The process offers a description of a given food system, detailing production, commercialization, storage and consumption of the food. It identifies the microbiological hazard(s) associated with the system, and provides information on the possible transfer of the hazard(s) to consumers to cause harm (Lammerding *et al.,* 2001).

Lammerding (1998) indicated that MRA has unique features that differentiate it from chemical risk assessments. For example, unlike chemical risk assessments that may consider the cumulative effects of carcinogens and other toxicants causing chronic effects, MRA focuses on outcomes that are primarily the result of single exposures. Each exposure to a pathogen or its toxin represents an independent, non-cumulative event, resulting in outcomes ranging from asymptomatic infection to acute illness, chronic syndromes, or death.

The outcomes of MRA serve as tools that are used to address the health and safety challenges presented by the identified food-borne hazard.

2.10.1.1 Sample microbial risk assessments (MRA)

A number of major risk assessments have been conducted at country and international levels by individuals, governments and intergovernmental organizations. These include MRA for *Listeria monocytogenes* in ready-to-eat foods and deli meats, *Salmonella enteritidis* in eggs and egg products, *Vibrio parahaemolyticus* in oysters, Enterohemorrhagic *Escherichia coli* in ground beef, and Fluoroquinoline resistance in *Campylobacter* (FAO/WHO, 2004).

Lindqvist and Westoo (2000) conducted a quantitative risk assessment for *L. monocytogenes* in smoked salmon and rainbow trout in Sweden and found risks for listeriosis to be 2.0×10^{-3} for low-risk individuals and 1.6×10^{-2} for high-risk individuals.

Joint WHO/FAO risk assessments include *Salmonella enteritidis* in broilers and eggs, *Campylobacter sp* in broilers, and *L. monocytogenes* in ready to eat foods (Buchanan, 2003). In the *L. monocytogenes* risk assessments, the RTE foods examined were milk, ice cream, cold-smoked fish, and fermented meat products. The risks of listeriosis per serving were 5.0×10^{-9} for milk, 1.4×10^{-11} for ice cream, 2.1×10^{-8} for smoked fish, and 2.5×10^{-12} for fermented meats (WHO/FAO, 2004). Smoked fish thus recorded the highest risk per serving. However, relative to the other foods, the consumption of smoked fish was indicated to be modest, hence the estimated total number of listeriosis due to the product was moderate (WHO/FAO, 2004).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

The study comprised a survey and laboratory microbiological analyses. The survey solicited information on the consumption patterns (frequency and portion sizes) of hot-smoked fish (mackerel, tuna, and herrings), salted-dried tilapia (*koobi*), salted-dried ray fish (*kako*), salted and fermented fish (*momoni*), and sundried sardines among consumers in two coastal and an inland community in the Accra and Tema Metropolitan Assemblies to enable assessment of their exposure to *Listeria monocytogenes*. A total of 450 consumers, 150 each from Jamestown and Tema New Town (coastal communities) and Madina (inland community), were interviewed with semi-structured questionnaires (Appendix 1). Four (4) processors of each product type were also selected at processing sites and interviewed with semi-structured questionnaires (Appendix 2) on their processing methods and practices.

Laboratory microbiological analyses involved detection and enumeration of *Listeria monocytogenes* as well as determination of general microbial counts (total plate count, total coliform count, and *Escherichia coli* count) in fish samples collected from processing sites, some informal markets in Accra and Tema, and food vendors[1]. Additionally, the ability of *L. monocytogenes* to survive domestic cooking was investigated in a challenge test, in which hot-smoked fish (tuna, herrings and mackerel), sundried sardines, and *koobi* were inoculated with *Listeria monocytogenes* NCTC 11994 at $\geq 10^8$ CFU/ml in buffered peptone water, used to prepare some typical

[1] General microbial counts were not determined for samples from food vendors

Ghanaian soups and then tested for the presence/absence of the pathogen after cooking.

Standard tests (Board *et al*., 1992) were used to confirm all presumptive *L. monocytogenes* isolates. Aseptic procedures were followed during sampling and microbiological analyses.

Estimation of the risk of consumers ingesting *L. monocytogenes* through consumption of the traditionally processed fish was done using data on the counts of the pathogen in the fish products and survey data on the portion sizes of fish often consumed at an instance.

3.2. Overview of survey and sampling sites

Consumer and processor surveys were conducted in Jamestown and Tema New Town, two coastal fishing communities in the Accra and Tema Metropolitan Assembly respectively, known for their traditional fish processing activities, and in Madina, an inland community with a large central market patronized by several consumers in the Accra Metropolitan Assembly.

Fish samples were obtained from three (3) inland informal markets (Madina, Kaneshie and Agbogbloshie markets) and two in the coastal communities, Jamestown and Tema New Town. The markets were selected by convenience from the list of markets to which processors indicated they sent their products.

Foods served with smoked fish in soup were also purchased from the University of Ghana Night Market, a major eating joint for students and some staff in the University, to determine the occurrence of the pathogen at the point of consumption.

3.2.1 Survey

A total of 462 respondents consisting of 450 consumers and 12 processors were interviewed using pretested semi-structured questionnaires (Appendices 1 and 2).

3.2.1.1 Consumer survey

The number of consumers, n, was determined using the following formula from McCabe and Moore (1993):

$$\mathbf{n= (Z_{\alpha/2}/2m)^{2}}$$

where $Z_{0.025}$= 1.96, m (margin of error)= 5%, α= 0.05 C.I= 95%

Using the indicated margin of error, m, level of significance, α, and confidence interval, CI, n was calculated as:

$$n = [(1.96/0.1)^2]$$

$$n = 19.6^2 \approx 20^2$$

$$n = 400$$

This number (n=400) was increased to 450 in order that 150 respondents each could be drawn from the three communities (Jamestown, Tema New Town, and Madina). The inclusion criteria were that prospective respondents be consumers of traditionally processed fish, be involved in the preparation and/or distribution of food in their households, and be able to estimate the portion sizes of the fish products often consumed by children aged 6 months to 6 years, the elderly (≥60 years), and pregnant women, if they had any of such persons in their households at the time of the survey.

To aid the estimation of portion sizes, packaged 50g samples of sundried sardines, smoked fish (tuna, mackerel, and herrings), and 10g samples of *kako* and *koobi* were shown to respondents. They were then asked to use those as models to estimate the quantities of the products they often consumed at an instance as multiples or fractions of the sizes shown them. A mould of plasticine (artificial clay) representative of 10g of *momoni* was used as a model for that product. Where respondents had children, the elderly and/or pregnant women in their households they were asked to make proxy estimations of the frequencies and portion sizes of the traditionally processed fish those individuals often consumed.

3.2.1.2 Processor survey

Four (4) processors each of smoked fish, salted fish, and sun-dried sardines were selected by convenience and interviewed with semi-structured questionnaires on their methods of processing and general fish handling practices. The interviews were conducted at the processing sites to enable observation of the methods and practices they describe.

3.3 Sampling for microbiological analyses

Four categories of samples were collected for laboratory microbiological analyses: fish from informal market; fish and water from some steps along the processing chains of the various products; freshly processed hot-smoked fish for the challenge test; and hot-smoked fish served with food from food vendors.

Samples collected were appropriately labelled and packed into thermos ice chest previously sanitized with 70% ethanol, and transported to the laboratory on ice for immediate analysis

3.3.1 Sampling of fish from informal markets

Fifteen (15) samples of each fish product were purchased from Madina, Kaneshie, Agbogbloshie, Jamestown, and Tema New Town markets (total of 105 samples). On each sampling day, fish were purchased from the markets as typically sold to consumers.

3.3.2 Sample collection from processing Sites

Fish and water samples (total 64) were collected from some steps along the traditional fish processing chain as indicated in Table 3.1. Gloves were worn in the process to prevent confounding contamination.

Table 3.1: Sampling points along traditional fish processing chain

Product	Sampling point/sample type	Number of samples
Momoni	• Fresh fish before washing	2
	• Fresh fish after washing	2
	• Fish after three days soaking in highly saturated salt solution	2
	• Water before use for washing	2
	• Water after use for washing	2
	• Fish after three days sun drying	2
Kako	• Fresh ray fish before washing	2
	• Fresh ray fish after washing	2

Table 3.1 continued

Kako	• Water before use for washing	2
	• Water after use for washing	2
	• Ray fish after three days soaking in highly saturated salt solution	2
	• Fish after three days sun drying	2
Smoked Tuna	• Fresh tuna before washing	2
	• Fresh tuna after washing	2
	• Water before use for washing	2
	• Water after use for washing	2
	• Tuna after smoking	2
Smoked Mackerel	• Fresh mackerel before washing	2
	• Fresh mackerel after washing	2
	• Water before use for washing	2
	• Water after use for washing	2
	• Mackerel after smoking	2
Smoked Herrings	• Fresh herring before washing	2
	• Fresh herring after washing	2
	• Water before use for washing	2
	• Water after use for washing	2
	• Mackerel after smoking	2
Sundried sardines	• Fresh sardines before washing	2
	• Sardines after washing	2
	• Water before use for washing	2
	• Water after use for washing	2
	• Sardines after three days drying	2
	Total	**64**

3.3.3 Sample collection for challenge test

Hot-smoked tuna, mackerel and herrings were purchased from fish processors in Tema New Town immediately after processing (while still hot). The fish were collected directly from the smokers into sterile sample bags. *Koobi* and sundried sardines were purchased from the Madina market as typically sold to consumers.

3.3.4 Sample collection from food vendors

Hot-smoked tuna, mackerel and herrings were purchased with soup and *banku* (stiff porridge made from fermented corn dough and cassava dough) from food vendors at the University of Ghana Night Market as normally dished to consumers. The market is a major point for the sale of street foods to students and some staff of the University.

3.4 Laboratory microbiological analyses

Samples were analysed for the presence and concentration of *L. monocytogenes* using the United States Department of Agriculture protocols. Total plate count, total coliform count, and *Escherichia coli* counts were also determined for each sample except those purchased from food vendors.

3.4.1 Sample preparation and enrichment for *L. monocytogenes* detection and enumeration

Twenty-five grams (25g) of each field sample of fish was weighed into sterile stomacher bags. To this quantity, 225mL of Listeria Enrichment Broth (LEB) was

added, and the diluent-fish mixture homogenized with a stomacher blender (Seward Stomacher®400 Circulator) for 60 seconds. The homogenate was incubated at 30°C for 24±2 hours. For water samples, 25mL of water was added to 225mL of LEB. After the incubation period, 0.1mL of the primary enrichment broth was dispensed into 10mL of Fraser broth and incubated at 37°C for up to 48 hours as the secondary enrichment step.

3.4.2 Plating of enriched cultures

Using the spread plate technique, 0.1mL of the secondary enrichment broths were plated out on Oxford or Chromogenic agar plates and incubated aerobically at 37°C for up to 48 hours.

3.4.3 Enumeration

Aliquots (0.1mL) of 10^{-1} homogenates of fish sample units, prepared as previously described, were plated out using the spread plate technique on Oxford agar plates or Chromogenic agar plates. The plates, after adding the inocula, were allowed to stand for 15 minutes to allow absorption of the inocula into the agar before incubating at 37°C for 24 – 48 hours. The incubated plates were examined and typical colonies of *L. monocytogenes* (brownish colonies with black halos on Oxford agar and greenish colonies with whitish halo on Chromogenic agar) were counted using a Quebec colony counter. Plates containing up to 150 colonies were considered useful for enumeration.

3.4.4 Presumptive and confirmatory identification

Typical presumptive *L. monocytogenes* colonies were purified by streaking on nutrient agar and incubating the plates at 37^{o}C for 24 hours (Board *et al*., 1992). Purified colonies from the nutrient agar plates were tested for their Gram reaction within 24 hours of visible growth. The Gram staining procedure described by Black (1999) was employed. Colonies identified as pure by their Gram reaction were used for subsequent confirmatory identification tests.

3.4.4.1 Catalase test

The catalase test procedure described by Atlas (1997) was used. With a sterilized inoculation loop, pure colonies of *L. monocytogenes* were smeared on clean glass slides. Drops of 3% hydrogen peroxide were placed on the smears using a capillary pipette. The slides were then observed for gas bubbles.

3.4.4.2 Acid production from carbohydrates

The fermentation of D-mannitol, D-xylose, L-rhamnose, and D-glucose by the isolates were determined. One tube each of the four carbohydrates (at 5% concentration in purple broth base) was inoculated with pure isolates. Inoculated broths were incubated at 37^{o}C for up to 72 hours and observed for colour changes. Acid production manifests as a change in broth colour from purple to yellow (Board *et al*., 1992).

3.4.4.3 β-haemolysis test

Blood agar plates were streaked with presumptive *L. monocytogenes* isolates, incubated at 37^{o}C for 18 hours, and observed for clear bands around the lines of streak

(Board *et al*., 1992), (indicative of red blood cell haemolysis). Positive *L. monocytogenes* isolates showed narrow bands away from lines of growth (Board *et al*., 1992).

3.4.4.4 Test for umbrella-like growth in semi-solid agar

Sulphur Indole Motility (SIM) agar deep tubes were inoculated with presumptive *L. monocytogenes* by stabbing, incubated at 25^{o}C for up to 48 hours, and observed for a characteristic growth pattern described by Board *et al*., (1992) as high turbidity about 1cm below the meniscus and away from the stab line such that the turbidity has the appearance of an open umbrella.

3.5 Survival of *L. monocytogenes* in domestic cooking (challenge test)

The ability of *L. monocytogenes* to survive domestic cooking was tested by inoculating smoked fish (tuna, mackerel and herrings), *koobi*, and sundried sardines with *Listeria monocytogenes* NCTC 11994 and using the fish to prepare two kinds of soups commonly consumed in Ghanaian homes: one with a short cooking time (45 minutes; *light soup*) and another with a longer cooking time (75 minutes; groundnut soup). The composition of the soups (Table 3.2) and duration for cooking were determined through a focus group discussion with volunteers (five women with average age of 24 years) involved in food preparation in their homes. The discussion was held to determine the methods, average cooking times, and regular ingredients often used for preparing *light soup* and groundnut soup.

3.5.1 Inoculation of fish

A broth of *L. monocytogenes* NCTC 11994 was prepared by incubating microdiscs of the pathogen in buffered peptone water at 37^{o}C for 24 hours to amplify their numbers.

After incubation, the count of the pathogen was determined to ensure it was $\geq 10^8$ CFU/ml. The culture was then poured onto the respective fish products in separate sterile stomacher bags. The broth-fish mixtures were shaken by hand and incubated at 37^{o}C for 24 hours (a stomacher blender was not used to avoid breaking up the fish) to allow the pathogen to enter and grow in the fish tissues. The fish were used for preparing the soups after the incubation period.

3.5.2 Soup preparation

Two sets of soups were prepared; experimental set prepared with fish contaminated with *L. monocytogenes*, and control set prepared with uncontaminated fish. Cooking started at the same time for all the soups. Total cooking time for *light soup* was forty five (45) minutes and that for groundnut soup was seventy five (75) minutes. The soups were prepared by some respondents from the focus group discussion. The procedures used to prepare the soups are as shown in Figs 3.1 and 3.2. Based on the outcome of the focus group discussion, the kinds and quantities of ingredients in Table 3.2 were used for preparing the soups. The volume of water used for each soup was 1040 mL.

The control soups samples enabled evaluation of the sensory appeal of the soups cooked for the indicated durations. The *light soups* maintained an average temperature of 99.2^{o}C during cooking whilst the groundnut soups maintained an average temperature of 106.5^{o}C.

Table 3.2: Kinds and quantities of ingredients used for challenge test cooking

Light soup		Groundnut soup	
Ingredient	**Quantity (g)**	**Ingredient**	**Quantity (g)**
Tomatoes	74	Tomato paste	35
Garden eggs	171	Groundnut paste	150
Pepper	12	Pepper	5
Salt	7.5	Salt	7.5
Onion	61	Onion	50

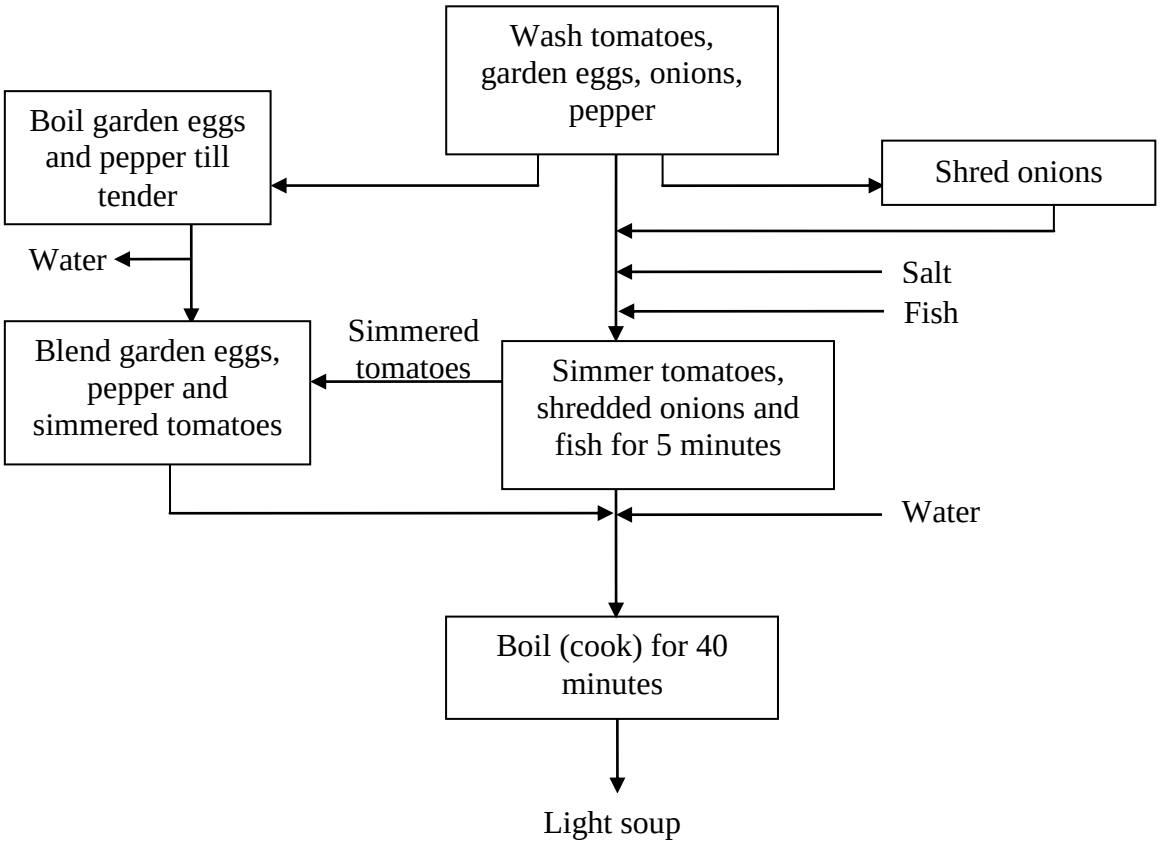

Fig. 3.1: Flow diagram for the preparation of light soup

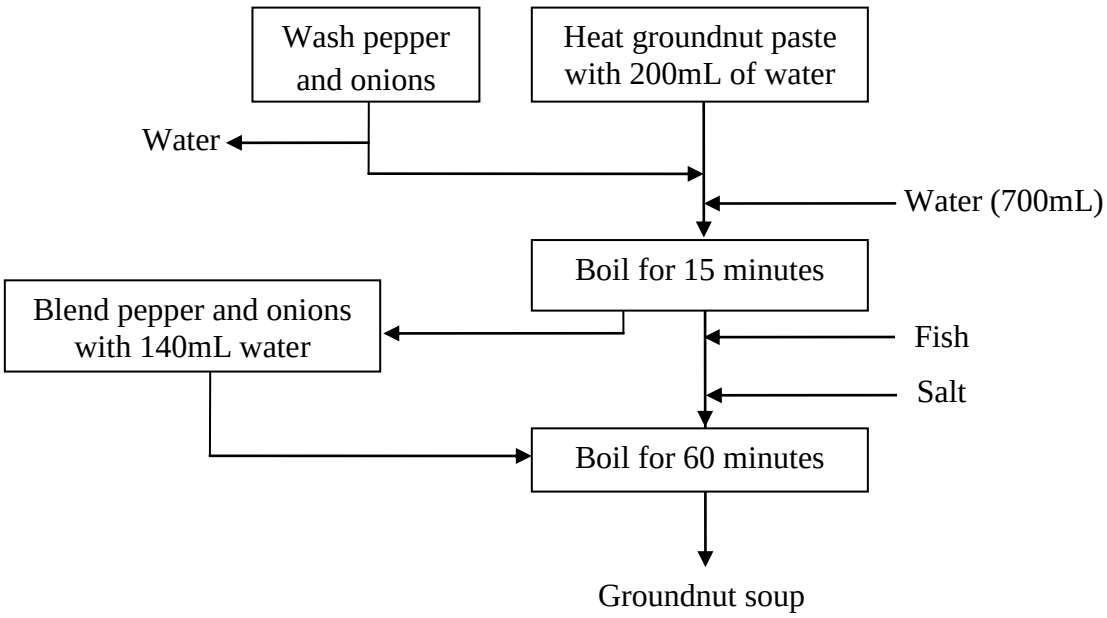

Fig. 3.2: Flow diagram for the preparation of groundnut soup

Immediately after cooking, the fish were removed from the soups and prepared for the detection of *L. monocytogenes* following the methods described in Section 3.4.

3.6 Quality control for microbiological analyses

Parallel tests for the identification were conducted on a positive control (*Listeria monocytogenes* NCTC 11994) and a negative control (*Enterococcus faecalis* NCTC 775) (HPA, 2009).

3.7 Microbial Risk Assessment Protocols

The Codex Alimentarius Commission (CAC, 2003) framework for risk assessment (Fig. 3.4) was used for the study. The hazard identification was done using available literature on the pathogen. Survey and laboratory analyses provided data for fulfilling the requirements of the remaining three steps of the Codex risk assessment protocol.

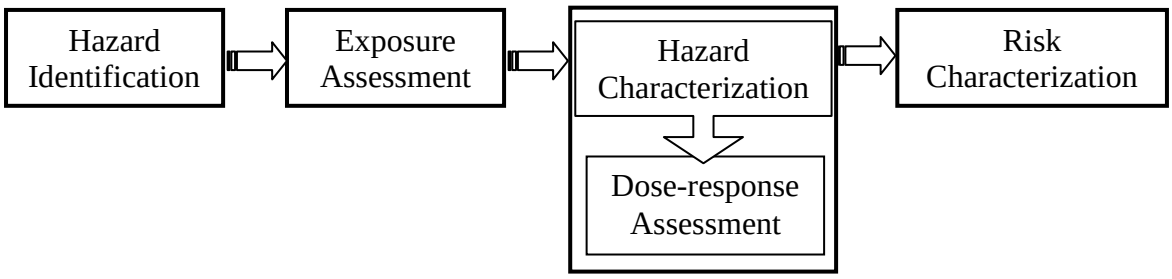

Fig. 3.3: General risk assessment framework (CAC, 1998)

3.7.1 Hazard identification

The identified hazard in this study was *Listeria monocytogenes*, a Gram positive, microaerophilic, non-spore forming psychrotoph on which epidemiological evidence abounds on its pathogenicity (Lindqvist and Westoo, 2000; Lammerding *et. al*, 2001).

3.7.2 Exposure assessment

The exposure of consumers to *L. monocytogenes* was assessed using data from the laboratory analyses and field survey to determine the **prevalence** and **concentration** of the pathogen in the fish, as well as the likely **intakes** of the pathogen.

3.7.2.1 Prevalence of *L. monocytogenes*

This was determined as the percentage of fish samples in which the pathogen was detected.

3.7.2.2 Concentration of *L. monocytogenes*

The concentrations of the pathogen were determined as the colony forming units of confirmed *L. monocytogenes* per gram of fish products.

3.7.2.3 Likely intakes of *L. monocytogenes*

The likely numbers of the pathogen in fish at the time of consumption, N, were estimated as

$$N = C \times S \text{ ----------(1)}$$

where N = likely number of *L. monocytogenes* cells ingested
C = CFU/g of *L. monocytogenes* in the fish product
S = serving size of fish product
(Lindqvist and Westoo, 2000)

3.7.3 Hazard characterization and dose-response assessment

There were two risk outputs in this study: risk of ingestion of the pathogen, and risk of infection with the pathogen. The risk of ingestion gave an indication of the probable intakes of the pathogen through the fish products, whereas the risk of infection provided the probability of occurrence of disease following ingestion of the pathogen. The risk of ingestion was determined using Equation 1 (Section 3.7.2.3).

The Weibull-Gamma dose-response model suggested by Farber *et al*., (1996), and used by Bemrah *et al*., (1998) to estimate the risk of listeriosis from consumption of soft cheese made from raw milk and Lindqvist and Westoo (2000) to estimate the risk of listeriosis from consumption of smoked salmon was used in the present study to provide estimations of the risk of infection. According to the model, the probability of illness from ingestion of *L. monocytogenes* is given by

$$P_{ill} = 1 - [1 + (N^b)/\beta]^{-\alpha} \text{ ----------(2)}$$

where P_{ill} = probability of illness
N = dose of *L. monocytogenes* (i.e. likely number ingested, from Equation 1)
α, β, b = model parameters
α=0.25, b=2.14 (Bemrah *et al*., 1998)
$\beta=10^{10.98}$ for high-risk population
$\beta=10^{15.26}$ for low risk population (Bemrah *et al*., 1998)

Although there are other models (such as the exponential model), the Weibull-Gamma model has the advantage of being particularly more suitable for risk assessments on *Listeria monocytogenes* (Lindqvist and Westoo, 2000; Bemrah *et al*., 1998; Farber *et al*., 1996) and is thus used more often. For example, a disadvantage of the exponential model not found with Weibull-Gamm is that the former overestimates risks (Lindqvist and Westoo, 2000).

3.7.4 Risk characterization

All the qualitative and quantitative information gathered in the previous steps were integrated to provide a scientifically sound description of the risk of ingesting *Listeria monocytogenes* through traditionally processed fish purchased from informal markets in Accra and Tema.

3.8 Data analyses

Survey data were analysed using Microsoft Excel and SPSS v16. The identity of *L. monocytogenes* isolates were established by comparison of the results of the preliminary and confirmatory tests with those obtained for the control organisms (*Listeria monocytogenes* NCTC 11994 and *Enterococcus feacalis* NCTC 775). Distributions for the risk of illness resulting from ingestion of *L. monocytogenes* were constructed using @Risk 5.5 to make up for uncertainties and provide risk estimations in other possible levels of contamination and fish serving sizes.

CHAPTER FOUR

4.0 RESULTS AND DATA ANALYSIS

4.1 Key findings

Consumer responses indicated that generally, smoked fish and salted fish were the most and least consumed products, respectively. For each product, consumption was reportedly higher in quantity and more frequent among adults (respondents, the elderly and pregnant women) than among children.

The hygienic conditions of processing sites and fish handling practices were generally unsatisfactory. Most sites were located close to areas of unsanitary conditions, including puddles of dirty water. Processors hardly washed their hands before processing, and wash water for raw fish was reused when it ideally needed to be changed.

Generally, the microbiological quality of fish improved through the processing steps to the finished products. For example, although *Listeria monocytogenes* and *Escherichia coli* were present in samples from the initial processing steps of smoking, they were not detected in samples collected immediately after smoking. However, in salted fish and sundried sardines, *E. coli* and *L. monocytogenes* were detected in some of finished product samples.

Although the prevalence of *L. monocytogenes* in the fish products from informal markets was high (40-80%), the counts were generally low (10^{2-3} CFU/g). Accordingly, the risks of ingestion and infection were found to be low.

4.2 Traditional fish processing

4.2.1 Demographic characteristics of processors

All processors were females aged 20 years or more (Fig. 4.1) who had received some level of formal education (Fig. 4.2). Each processor had been involved in the business for at least six years (Fig. 4.3).

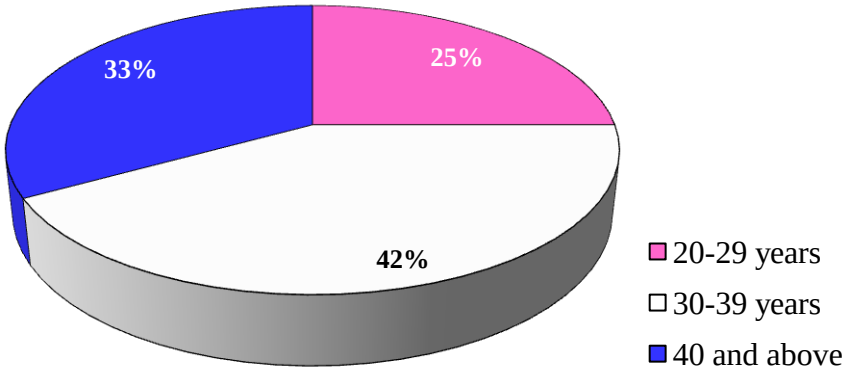

Fig 4.1: Age of traditional fish processors in Jamestown and Tema New Town

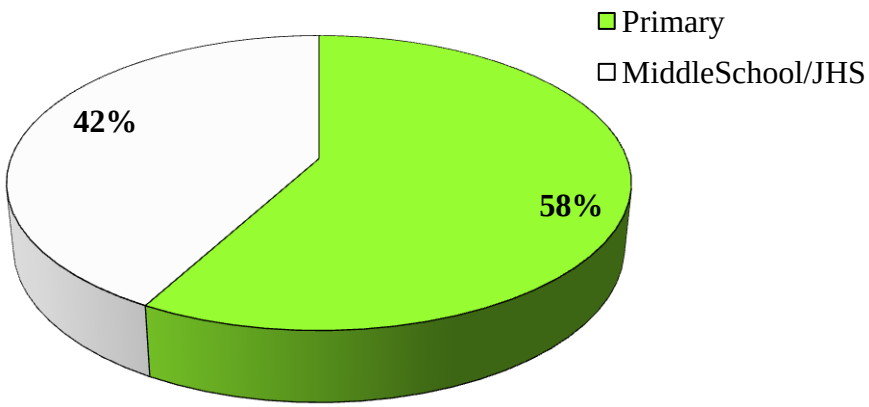

Fig 4.2: Highest level of education attained by traditional fish processors in Jamestown and Tema New Town

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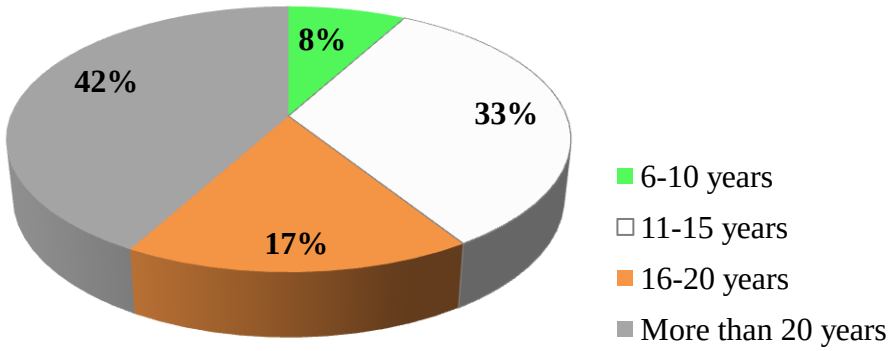


Fig. 4.3: Number of years in traditional fish processing

4.2.2 Processing methods

Traditional fish processing practices have not changed much over the years; the materials for and the means of processing observed in this study were not different from those reported earlier by Essuman (1982), Yankah (1988), Nketsia-Tabiri (1994), Cofie (2003) and Oppey (2002). The basic requirements for the processing of each fish product were fish, firewood, salt and sunlight. These were employed in different combinations for each product.

4.2.2.1 Procurement of fish

Fish were either purchased from fisher folk at the sea shores where fresh fish is landed or from cold stores (Table 4.1). Low premium, old stock and almost-stale fish were also purchased from cold stores for *momoni* processing. Fresh mackerel and herrings were purchased from cold stores as frozen fish whiles tuna, ray fish for *kako* and sardines for sun-drying were purchased from fishmongers from the shores. Purchased fish were transported to processing sites by foot, in taxis or by trolleys (Table 4.1).

Table 4.1: Purchasing of fresh fish for traditional processing

Item	Number of processors (%)
Source of fish (purchasing point)	
Fishmongers	12 (100)
Cold store	8 (67)
Inspection of fish before purchasing	12 (100)
Smell	8 (67)
Skin surface for sliminess	3 (25)
Duration of transportation to processing site	
Less than 30min	4 (33)
30min – 1 hr	6 (50)
1hr 30mins – 3 hrs	2 (17)
Means of transportation	
By foot	12 (100)
Trolleys	7 (58)
Commercial vehicles (taxi)	12 (100)

Cartons of frozen mackerel and herrings were mostly transported using taxi (or other commercial vehicles) whereas tuna, ray fish and sardines were carried in basins and transported by foot on head loads.

4.2.2.2 Smoking

Regardless of the kind of fish (i.e. tuna, mackerel or herrings), the method of smoking was essentially the same. The fish were washed, degutted, cut into three pieces if desired (head, mid-portion, tail), washed, arranged on a smoker and smoked for 2-3 hours for a soft product (i.e. wet hot-smoked product, tuna and mackerel) or ≥12 hours for a smoke-dried product (i.e. dry hot-smoked product, herrings) (Fig.4.4 and 4.5).

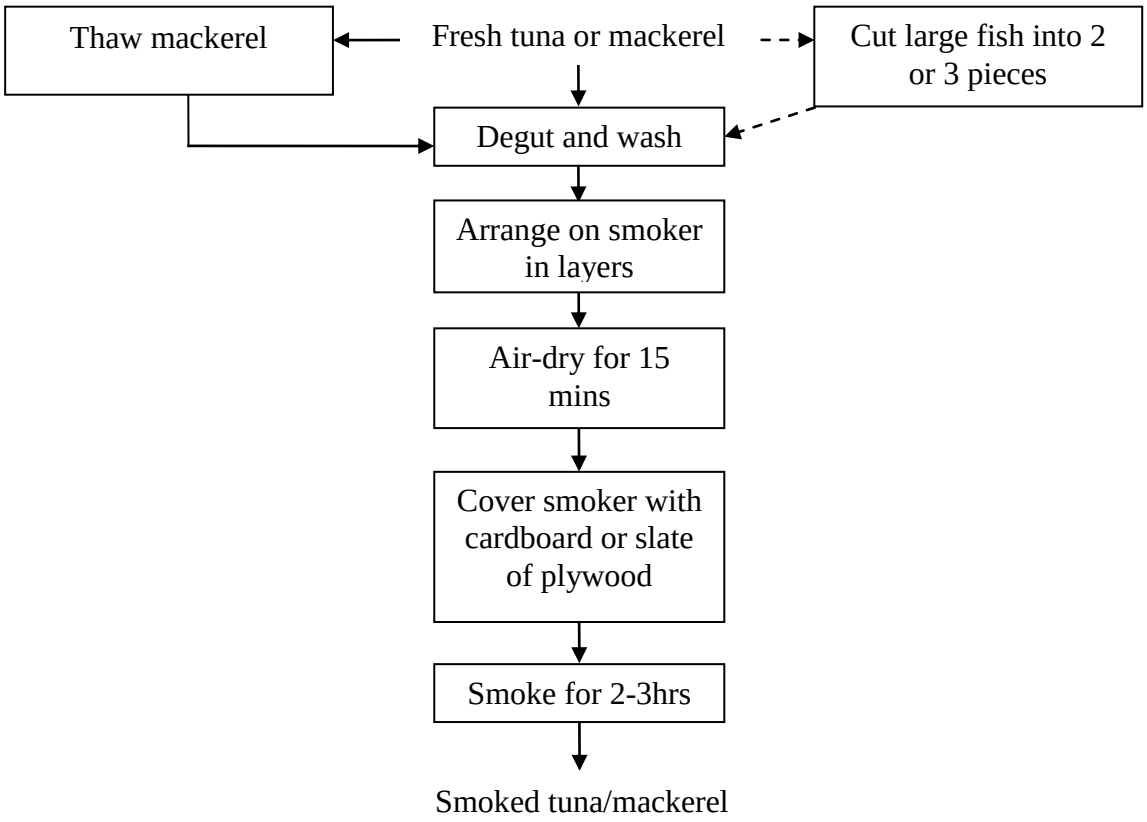


Fig 4.4: Process flow diagram for hot-smoked tuna and mackerel

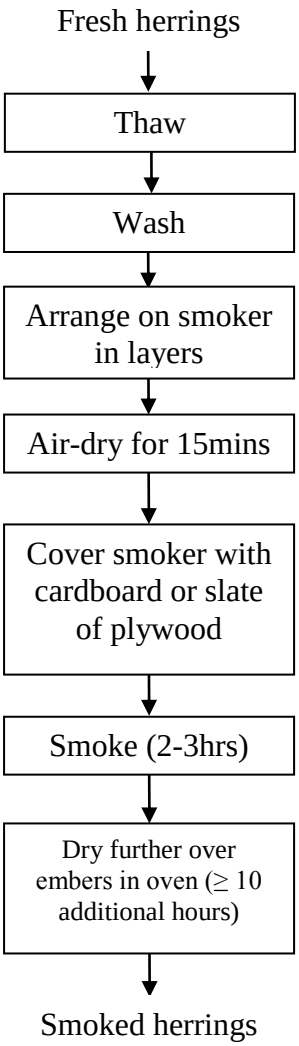


Fig. 4.5: Flow diagram for hot-smoked herrings

Tuna and mackerel were considered sufficiently smoked when the skin colour was golden-brown and the flesh tender whiles herrings were considered sufficiently smoked when fish were considerably brittle. Soon after processing, finished products were either retailed by hawking or sold in bulk. Entire batches of smoked tuna and salmon were usually sold on the same day of processing as there were no appropriate storage facilities. Smoked herrings (smoke-dried) could however be kept on the smoking racks or in baskets for up to five days or more until sold out.

Most processors used either metal ovens (Fig. 4.6) or concrete Chorkor smokers (Fig. 4.7).

Fig. 4.6: Metallic ovens

Fig. 4.7: Chorkor smoker

4.2.2.2.1 Hygiene of smoking environment

The hygienic conditions of the smoke sites were generally unsatisfactory. Some of the sites were close to unsanitary shores where human defecation was common, and to puddles of dirty water (Fig. 4.8). There were no sanitary facilities and pipeborne water. Additionally, the grounds were not cemented, facilitating possible transfer of dust and sand into fish handled close to the ground.

Note stagnant water (arrowed)

Note dirty gutter behind smoker (arrowed)

Fig. 4.8: Sanitation at smoking site

4.2.2.2.2 Handling practices during and after processing

Fish handling practices were also unsatisfactory. Although all processors indicated that they washed their hands before processing, the practice was not observed. Apart from the actual smoking, most of the unit operations were carried out very close to the bare ground which could expose the fish to microbial contaminations. For example, frozen fish were thawed on open cartons on the bare ground (Fig. 4.9). In most instances, some of the fish came into direct contact with the soil on the ground. Additionally, water used for washing fresh fish was not changed as often as it should have (Fig. 4.10).

Fig. 4.9: Thawing mackerel
Note spillage on ground

Fig. 4.10: Washing mackerel
Note colour of water used

Improper post-processing handling were also unhygienic. In some cases, freshly smoked fish were placed very close to the ground (Fig. 4.11.).

Fig. 4.11: Smoked mackerel ready for the market. Note nearness to bare ground

4.2.2.3 Sun-drying

Sun-drying was the method of choice for the traditional processing of sardines. The process involved the simplest of unit operations. Landed sardines were simply washed and spread out on the bare ground and left to dry for 3 - 5 days, depending on weather conditions. The dried fish were swept and gathered into heaps, collected in baskets and were ready for marketing.

4.2.2.3.1 Sanitary conditions of processing

It was observed that fresh sardines were not thoroughly washed, but were merely dumped into the wash water, scooped into baskets, and spread out on the bare ground. Wash water was obtained from the sea, and was used to wash several batches of fish without changing when it became necessary (Fig. 4.12).

The fish were dried on the bare ground which was not zoned off or protected, and were therefore exposed to several contaminants (Fig. 4.13). This was unsatisfactory, particularly since no subsequent step was available in the processing flow to eliminate any pathogenic contaminations that could occur through contact of the fish with the ground.

Fig. 4.14 shows how sardines were collected after drying. Note person walking on drying grounds. This was a common practice since the drying grounds were not zoned off.

Fig. 4.12: Washing fresh sardines. Note colour of water

Fig. 4.13: Spreading washed sardines on the ground to dry

Fig. 4.14: Sweeping dried sardines off the ground

Fig. 4.15: Dried sardines collected in a basket

4.2.2.4 Salting

Both *kako* (salted and dried ray fish) and *momoni* (salted and fermented fish) were processed essentially the same way. Fish were washed, salted for days under weights in salting vats (during which time some fermentation occurred in *momoni* processing) and sundried.

4.2.2.4.1 *Momoni* processing

The process flow diagram for *momoni* is as shown in Fig. 4.16. Stale fish purchased from cold stores were used for *momoni* (Fig. 4.17).

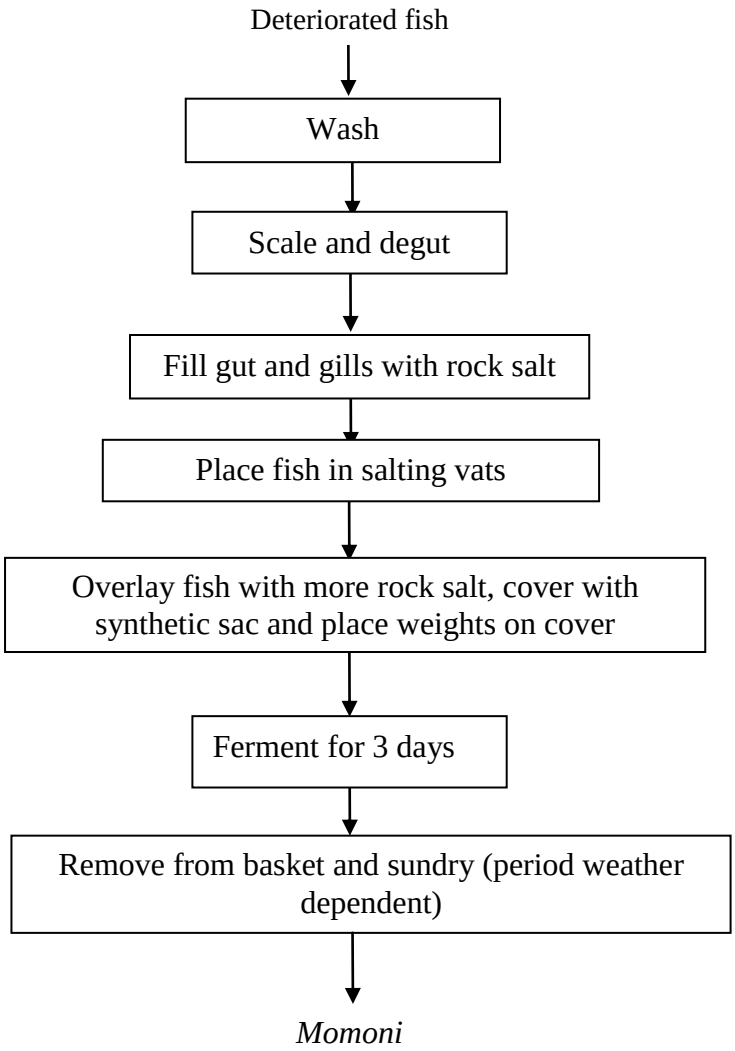


Fig. 4.16: Process flow for the production of *momoni*

4.2.2.4.1.1 Hygienic conditions of processing

As observed in traditional smoking and sun-drying, wash water were in similar unsatisfactory hygienic conditions. Fish were either dried directly on the bare ground,

or on nylon nets spread on the ground. The latter means did not seem to offer any prevention of fish contact with the soil. Hygiene of post-processing handling was poor (Fig. 4.18).

Fig. 4.17: Stacks of stale fish being moved from a cold store for *momoni* processing

Fig. 4.18: Gathering sufficiently dried *momoni.* Note processor stepping on fish (arrowed)

4.2.2.4.2 *Kako* processing

Ray fish were cut into chunks (Fig. 4.19), washed, slit and filled with salt, arranged in salting vats and sundried (Fig. 4.20). Fig. 4.21 illustrates the processing of *kako*.

Fig. 4.19: Cutting up ray fish

Fig. 4.20: Chunks of ray fish being sun-dried after salting

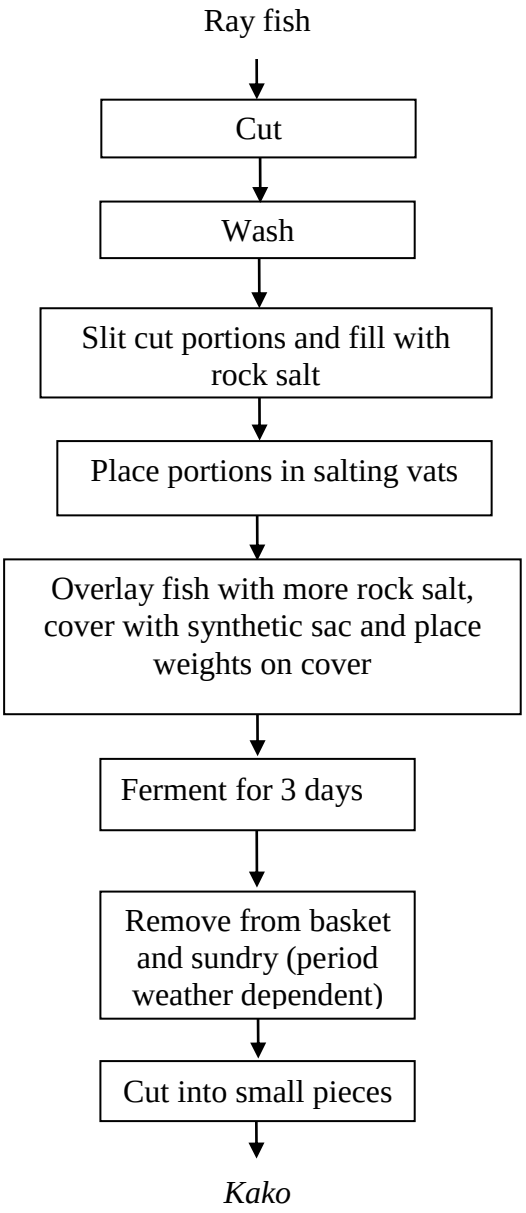


Fig. 4.21: Processing flow diagram for *kako*

4.3 Occurrence of *Listeria monocytogenes* and other microorganisms in fish during traditional processing

Generally, the microbiological quality of fish improved through the processing stages to the final products (Tables 4.2 – 4.5). In fresh tuna, ray fish, fresh sardines and fish for *momoni* processing, the average total plate counts ranged from $7.2x10^6$–$1.24x10^7$ CFU/g. Average total coliform counts ranged from $3.0x10^6$ – $8.5x10^6$ CFU/g. No microbial growth was recorded for frozen fresh mackerel and herrings. However, *L.*

monocytogenes and the other microorganisms tested for in the study were detected in all other fresh fish samples (Tables 4.2 – 4.5).

The microbiological quality of water used in processing was poor, as it contained high counts of total coliforms and *E. coli* (Tables 4.2 – 4.5). *L. monocytogenes* was also detected in all water samples.

In finished products, the average total plate counts ranged from 0 – $2.7x10^6$ CFU/g, whereas average total coliform and *E. coli* counts ranged from 0 – $1.90x10^6$ CFU/g and 0-$2.6x10^6$ CFU/g, respectively. *L. monocytogenes* was not detected in any of the smoked fish sampled immediately after processing, but was found in some of the other products (Tables 4.2 – 4.5).

Table 4.2: Average microbial counts (x10^5 CFU/g) and detection of *L. monoctyogenes* in samples along smoked fish processing chain in Tema New Town

	Tuna				Mackerel				Herrings			
Sample	**TPC**	**TCC**	**E. coli**	**Lm**	**TPC**	**TCC**	**E. coli**	**Lm**	**TPC**	**TCC**	**E. coli**	**Lm**
Fresh fish	113	64	24	+	nd	nd	nd	nd	nd	nd	nd	nd
Fish after washing	90	40	19	+	12	13	6	+	21	13	6	+
Water before washing	33	30	23	+	34	22	22	+	30	21	12	+
Water after washing	54	65	31	+	55	45	33	+	44	24	10	+
Fish after smoking	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd: not detected

TPC: total plate count

Lm: *Listeria monocytogenes*

+: detected

Note: Counts are averages. Actual values are presented in Appendix 4

Table 4.3: Average microbial counts and detection of *L. monoctyogenes* in samples along salted fish processing chain in Tema New Town

Sample	*Momoni*				*Kako*			
	TPC	**TCC**	**E. coli**	**Lm**	**TPC**	**TCC**	**E. coli**	**Lm**
Fresh fish	124	85	47	+	129	84	65	+
Fish after washing	119	63	29	+	106	63	51	+
Water before washing	84	38	18	+	59	33	22	+
Water after washing	151	73	23	+	95	98	53	+
Fish after salting and fermenting	0	0	0	+	0	0	0	+
Fish after sun-drying	16.5	11	8	+	19	5	3	+

nd: not detected **TPC**: total plate count $x10^5$ CFU/g or ml **TCC**: total coliform count $x10^2$ CFU/g or ml, **E. coli**: E. coli count $x10^2$ CFU/g or ml **Lm**: *Listeria monocytogenes* +: detected

Note: Counts are averages. Actual values are presented in Appendix 4

Table 4.4: Average microbial counts and detection of *L. monocytogenes* in samples along sun-drying processing chain in Tema New Town

Sample	TPC	TCC	E. coli	Lm
Fresh fish	72	30	26	+
Tuna after washing	57	28	26	+
Water before washing	31	18	5	+
Water after washing	78	21	14	+
Fish after sun-drying	27	19	26	+

nd: not detected **TPC**: total plate count $x10^5$ CFU/g or ml **TCC**: total coliform count $x10^2$ CFU/g or ml, **E. coli**: E. coli count $x10^2$ CFU/g or ml **Lm**: *Listeria monocytogenes* +: detected

Note: Counts are averages. Actual values are presented in Appendix 4

Table 4.5: Average microbial counts and detection of *L. monoctyogenes* in samples along smoked fish processing chain in James Town

Sample	Tuna				Mackerel				Herrings			
	TPC	**TCC**	**E. coli**	**Lm**	**TPC**	**TCC**	**E. coli**	**Lm**	**TPC**	**TCC**	**E. coli**	**Lm**
Fresh fish	94	45	17	+	nd	nd	nd	nd	nd	nd	nd	nd
Tuna after washing	83	31	16	+	6	3	2	nd	12	6	2	+
Water before washing	44	26	15	+	35	18	12	+	31	14	8	+
Water after washing	56	48	27	+	43	26	24	+	37	22	10	+
Fish after smoking	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd: not detected **TPC**: total plate count $x10^5$ CFU/g or ml **TCC**: total coliform count $x10^2$ CFU/g or ml, **E. coli**: E. coli count $x10^2$ CFU/g or ml **Lm**: *Listeria monocytogenes* +: detected

Note: Counts are averages. Actual values are presented in Appendix 4

4.4 Occurrence of *Listeria monocytogenes* and other microorganisms in fish sold at informal markets

Listeria monocytogenes, *Escherichia coli* and total coliforms were detected at varying levels in fish samples purchased from the markets (Table 4.6). The trend in order of decreasing counts was total plate count > total coliform count > *E. coli* count > *L. monocytogenes* count. Generally, the highest counts of *L. monocytogenes* occurred in salmon and tuna, and the least counts in the salted fish products and sundried sardines.

In some fish samples (especially salted fish and sundried fish), *L. monocytogenes* was not detected by enumeration (direct culturing without preliminary enrichment) but was detected in the same samples plated out after primary and secondary enrichment in Listeria Enrichment Broth and Fraser Broth respectively (Table 4.6).

4.5 Detection of *Listeria monocytogenes* in street food samples

Listeria monocytogenes was not detected in any of the three street food samples (*banku* with groundnut soup). Food vendors kept soups containing the fish constantly on fire during sales. This was done to ensure that soups were hot to meet consumer preference, as, generally, consumers in the University of Ghana would not patronize the foods if they are not hot.

Table 4.6: Detection and average counts of *Listeria monocytogenes* and average general microbiological counts in fish samples purchased from informal markets in Accra and Tema

MKT	Count	Tuna	Mackerel	Herrings	*Kako*	*Koobi*	*Momoni*	Sundried sardines
Madina	**TPC** ($x10^5$ CFU/g)	87	76	47	26	21	43	76
	TCC ($x10^4$ CFU/g)	39	30	35	12	11	13	52
	E. coli ($x10^3$ CFU/g)	25	24	27	7	4	3	31
	Lm ($x10^2$ CFU/g)	21	29	8	7	6	3	3
	Lm Det	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ + +
Kaneshie	**TPC** ($x10^5$ CFU/g)	89	86	81	58	57	37	97
	TCC ($x10^4$ CFU/g)	49	35	41	22	20	20	50
	E. coli ($x10^3$ CFU/g)	20	22	25	11	11	12	25
	Lm ($x10^2$ CFU/g)	17	16	0	8	9	5	nd
	Lm Det	+ + +	+ + +	+ +	+	+ +	+ +	+ +
Agbogbloshie	**TPC** ($x10^5$ CFU/g)	82	71	48	34	24	22	42
	TCC ($x10^4$ CFU/g)	31	28	55	12	10	8	89
	E. coli ($x10^3$ CFU/g)	30	23	14	1	0	0	80
	Lm ($x10^2$ CFU/g)	8	10	2	1	0	0	0
	Lm Det	+ +	+ + +	+ +	+ +	+	+	+
Jamestown	**TPC** ($x10^5$ CFU/g)	83	75	68	26	23	27	74
	TCC ($x10^4$ CFU/g)	23	40	25	20	17	20	47
	E. coli ($x10^3$ CFU/g)	19	18	19	9	9	6	18
	Lm ($x10^2$ CFU/g)	12	9	9	1	2	2	2
	Lm Det	+ + +	+ + +	+ +	+	+	+ +	+
Tema NT	**TPC** ($x10^5$ CFU/g)	72	79	50	28	15	15	80
	TCC ($x10^4$ CFU/g)	39	43	23	14	3	11	43
	E. coli ($x10^3$ CFU/g)	39	28	17	2	1	0	33
	Lm ($x10^2$ CFU/g)	2	4	3	1	0	0	3
	Lm Det	+	+ +	+	+	nd	+	+ +

TPC: total plate count **nd**: not detected
TCC: total coliform count
Lm: *Listeria monocytogenes* count
Lm Det: Detection of *L. monocytogenes* by enumeration
+: *L. monocytogenes* detected in one sample by enrichment, 0 CFU/g recorded in two samples
+ +: *L. monocytogenes* detected in two samples by enrichment, 0 CFU/g recorded in one sample
+ + +: *L. monocytogenes* detected in three samples by both enrichment and enumeration
Tema NT: Tema New Town
MKT: Market

4.6 Challenge test

Table 4.7 shows that *Listeria monocytogenes* NCTC 11994 survived in *koobi,* sun-dried fish and in smoked fish (tuna, mackerel and herrings) used in the experimental cooking.

Table 4.7: Survival of *Listeria monocytogenes* NCTC 11994 in fish used in experimental domestic cooking

		Experiment		
Soup	**Fish**	**1**	**2**	**3**
Groundnut soup	Smoked tuna	+	+	nd
	Smoked mackerel	nd	+	+
	Smoked herrings	+	nd	nd
	Koobi	+	+	np
	Sun-dried sardines	+	+	nd
Light soup	Smoked tuna	+	+	+
	Smoked mackerel	nd	+	nd
	Smoked herrings	+	nd	+
	Koobi	nd	+	np
	Sun-dried sardines	+	nd	nd

+ : *L. monocytogenes* detected in fish **nd**: not detected **np**: not plated (fish broke up in soup)

4.7 Characterization of isolates

The characteristics of the presumptive *L. monocytogenes* isolates (Brownish/grey colonies with black halo on Oxford Agar Plates (Fig. 4.23) or greenish colonies with pale yellow halo on Chromagar plates) are as summarized in Table 4.8. Colonies with a green metallic sheen on Eosin Methylene Blue incubated at 44.5^{o}C for 24 hours were considered presumptive for *E. coli* (Fig. 4.24).

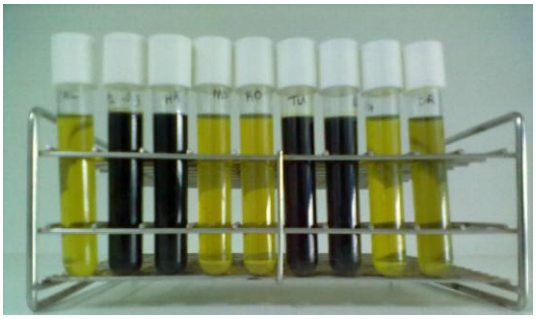
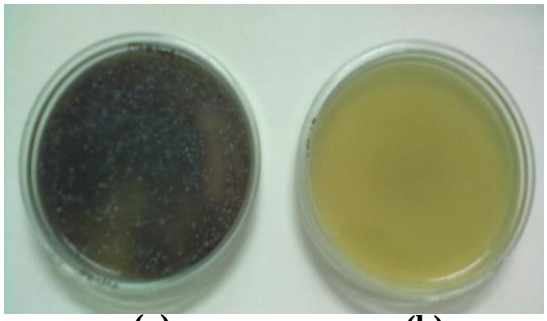


Fig. 4.22: Fraser broths after 24h incubation. Black tubes may contain *Listeria* species, yellow tubes do not



(a) (b)

Fig. 4.23: Presumptive *L. monocytogenes* colonies on Oxford Agar Plate (a), and a control Oxford Agar Plate (b)

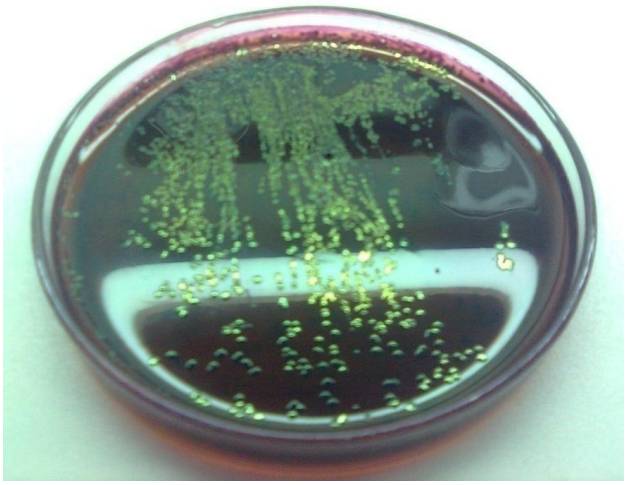


Fig. 4.24: *Escherichia coli* isolates on eosin methylene blue agar plate

Table 4.8: Characteristics of presumptive *Listeria* isolates

Isolate	Gram reaction, shape	Catalase reaction	Sugar Fermentation				Umbrella motility	β -H	Presumptive organism	Confirmed organism
			D-Glucose	Mannitol	Xylose	Rhamnose				
P001	+, rods	+	+	+	+	-	+	+	*L. monocytogenes*	*L. monocytogenes*
P002	+, rods	+	+	+	-	-	-	-	*Listeria* sp.	
P003	+, rods	+	+	+	-	-	-	-	*Listeria* sp.	
P004	+, rods	+	+	-	+	-	-	-	*Listeria* sp.	
P005	+, rods	+	+	-	-	-	-	-	*Listeria* sp.	

β – H: β haemolysis **+**: sugar fermented **-** :sugar not fermented
P001 – 005: *Listeria* sp. isolates 1 – 5.

4.8 Demographic characteristics of respondents

Table 4.9 presents a summary of the demographic characteristics of respondents. 'Respondents' as used in subsequent text refers to individuals actually interviewed in households visited. In addition to providing information about their own consumption patterns, the respondents made proxy estimations of the frequencies of consumption of traditionally processed fish, and the quantities thereof, by the elderly (≥60 years old), children (6months – 6 years), and pregnant women in their households.

4.9 Consumption of traditionally processed fish

Generally, smoked fish products were consumed more often and in greater quantities than sun-dried sardines and the salted fish products (*momoni* and *koobi*) in all the communities visited. Sections 4.9.1 – 4.9.2 show the respective consumption patterns in Jamestown, Tema New Town and Madina.

4.9.1 Jamestown

In Jamestown, among the smoked fish, mackerel and herrings were the most and least frequently consumed by the respondents, respectively. Frequency of consumption of *kako* was highest among the salted fish products. The summaries of the frequencies of consumption and quantities most often consumed at an instance are presented in Table 4.10. The respective frequencies and quantities of consumption of the products by respondents and the reported consumption patterns among the elderly, children, and pregnant women are presented in Appendix 3.

Table 4.9: Summary of demographic characteristics of respondents

	Age					Gender		Elderly (≥60 yrs) present in household		Pregnant woman present in household		Children (6mo-6yrs) present in household	
Community	**a**	**b**	**c**	**d**	**e**	**Male**	**Female**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Tema New Town	1	44	29	11	15	26	74	70	30	33	67	64	36
Jamestown	6	39	33	13	9	37	63	68	32	39	61	62	38
Madina	4	27	35	13	21	23	77	53	47	31	69	75	25

yrs = years old
mo = months old
a = Less than 20 yrs
b = 20 – 29 yrs
c = 30 – 39 yrs
d = 40 – 40 yrs
e = 50 yrs or more
All values represent percentages of respondents

Table 4.10: Summaries of highest frequencies of consumption of traditionally processed fish and highest quantities of the products consumed at an instance in Jamestown

	Product	Highest Frequency (%)	Most frequently consumed quantities (%) *
Respondents	Smoked tuna	2-3 times a week (70)	More than 200g (63.3%)
	Smoked mackerel	2-3 times a week (60)	151-200g (40)
	Smoked herrings	Once a week (50.7)	151-200g (58.7)
	Sundried sardines	Once a month	101-150g (40)
	Kako	Once a month	5-10g (53.7)
	Momoni	Once a week (48.7)	5-10g (52)
	Koobi	Once a month (65.3)	41-50g (45.2)
Elderly	Smoked tuna	Daily (52)	151-200g (35.6)
	Smoked mackerel	2-3 times a week (51.9)	151-200g (69.2)
	Smoked herrings	Once a week (76.3)	101-150g (44.2)
	Sundried sardines	Once a month (43.3)	101-150g (49)
	Kako	Never (68.3)	5-10g (51.5)
	Momoni	Never (66)	5-10g)51)
	Koobi	Never (63.5)	21-30g (60.5)
Pregnant women	Smoked tuna	Daily (58.6)	More than 200g (79.3)
	Smoked mackerel	2-3 times a week (60.3)	151-200g (32.8)
	Smoked herrings	Once a week (58.6)	101-150g (48.3)
	Sundried sardines	Once a week (58.6)	151-200g (55.1)
	Kako	Once a week (69)	5-10g (52)
	Momoni	Once a week (93.1)	5-10g (38.9)
	Koobi	Once a month (77.6)	More than 50g (48.3)
Children	Smoked tuna	2-3 times a week (76.3)	101-150g (73.2)
	Smoked mackerel	2-3 times daily (66)	101-150g (63.9)
	Smoked herrings	Once a week	50-100g (74.2)
	Sundried sardines	Once a month (84.5)	50-100g (100)

*** figures in parenthesis represent percentages of consumers**

4.9.2 Tema New Town

Similar to the consumption pattern in Jamestown, generally, smoked fish were consumed more frequently and in greater quantities than the dried and salted fish products. Table 4.11 summarizes the highest frequencies and quantities of consumption of the respective traditionally processed fish in Tema New Town. Individual differences in the actual frequencies and quantities of consumption of the products among respondents and values of the same reported for the elderly, children, and pregnant women are presented in Appendix 3.

4.9.3 Madina

On the whole, Madina recorded the least frequencies of consumption of the traditionally processed fish. The pattern of consumption was however similar to those reported for Jamestown and Tema New Town; smoked fish was consumed more frequently and in greater quantities whilst salted fish was the least consumed (Table 4.12). The respective quantities and frequencies of consumption of the fish products are shown in Appendix 3.

Table 4.11: Summaries of highest frequencies of consumption of traditionally processed fish and highest quantities of the products consumed at an instance in Tema New Town

	Product	Highest Frequency (%)	Most frequently consumed quantities (%) *
Respondents	Smoked tuna	2-3 times a week (76)	More than 200g (51)
	Smoked mackerel	2-3 times a week (62)	More than 200g (45)
	Smoked herrings	Once a week (51)	101-150g (43)
	Sundried sardines	Once a month (53)	101-150g (37)
	Kako	Once a month (32)	5-10g (40)
	Momoni	Once a week (57)	11-20g (35)
	Koobi	Once a month (54)	41-50g (37)
Elderly	Smoked tuna	2-3 times a week (76)	151-200g (42)
	Smoked mackerel	2-3 times a week (59)	151-200g (58)
	Smoked herrings	Once a week (49)	101-150g (44)
	Sundried sardines	Once a week (41)	101-150g (57)
	Kako	Never (65)	5-10g (50)
	Momoni	Once a month (33)	5-10g (52)
	Koobi	Never (65)	21-30g (63)
Pregnant women	Smoked tuna	2-3 times a week (68)	More than 200g (60)
	Smoked mackerel	2-3 times a week (72)	More than 200g (54)
	Smoked herrings	Once a week (56)	101-150g (52)
	Sundried sardines	Once a week (54)	101-150g (46)
	Kako	Once a week (74)	5-10g (78)
	Momoni	Once a week (96)	5-10g (44)
	Koobi	Once a month (52)	More than 50g (48)
Children	Smoked tuna	2-3 times a week (76)	101-150g (73)
	Smoked mackerel	2-3 times a week (66)	101-150 (64)
	Smoked herrings	Once a week (76)	50-100 (74)
	Sundried sardines	Once a month (84)	50-100g (100)

*** figures in parenthesis represent percentages of consumers**

Table 4.12: Summaries of highest frequencies of consumption of traditionally processed fish and highest quantities of the products consumed at an instance in Madina

	Product	Highest Frequency (%)	Most frequently consumed quantities (%)*
Respondents	Smoked tuna	Once a week (46)	More than 200g (70)
	Smoked mackerel	2-3 times a week (48)	More than 200g (47)
	Smoked herrings	Once a month (63)	101-150g (58)
	Sundried sardines	Once a month (83)	101-150 (43)
	Kako	Once a month (47)	5-10g (51)
	Momoni	Once a month (63)	41-50g (36)
	Koobi	Never (45)	5-10g (67)
Elderly	Smoked tuna	Once a week (66)	151-200g (46)
	Smoked mackerel	Once a week (71)	151-200g (68)
	Smoked herrings	Once a week (64)	101-150g (48)
	Sundried sardines	Once a month (51)	101-150g (46)
	Kako	Never (77)	11-20g (66)
	Momoni	Never (59)	21-30g (58)
	Koobi	Never (73)	5-10g (57)
Pregnant women	Smoked tuna	Once a week (76)	More than 200g (78)
	Smoked mackerel	2-3 times a week (72)	151-200g (48)
	Smoked herrings	Once a week (100)	151-200g (41)
	Sundried sardines	Once a month (74)	50-100g (70)
	Kako	Once a month (48)	5-10g (53)
	Momoni	Once a month (91)	More than 50g (72)
	Koobi	Once a week (50)	5-10g (67)
Children	Smoked tuna	Once a week (44)	50-100g (66)
	Smoked mackerel	Once a week (71)	50-100g (75)
	Smoked herrings	Once a month (63)	50-100g (79)
	Sundried sardines	Once a month (51)	50-100g (97)

*** figures in parenthesis represent percentages of consumers**

4.10 Exposure assessment for *Listeria monocytogenes*

4.10.1 Risk pathways and event trees

The points along the fish processing and/or distribution chain at which the *Listeria monocytogenes* could occur were determined to establish the possible route through which the pathogen is transferred to consumers (Figs. 4.25 – 4.27, in which **Blue text**: possible point of contamination **Green text**: possible point of pathogen elimination **Red text**: risk of ingestion)

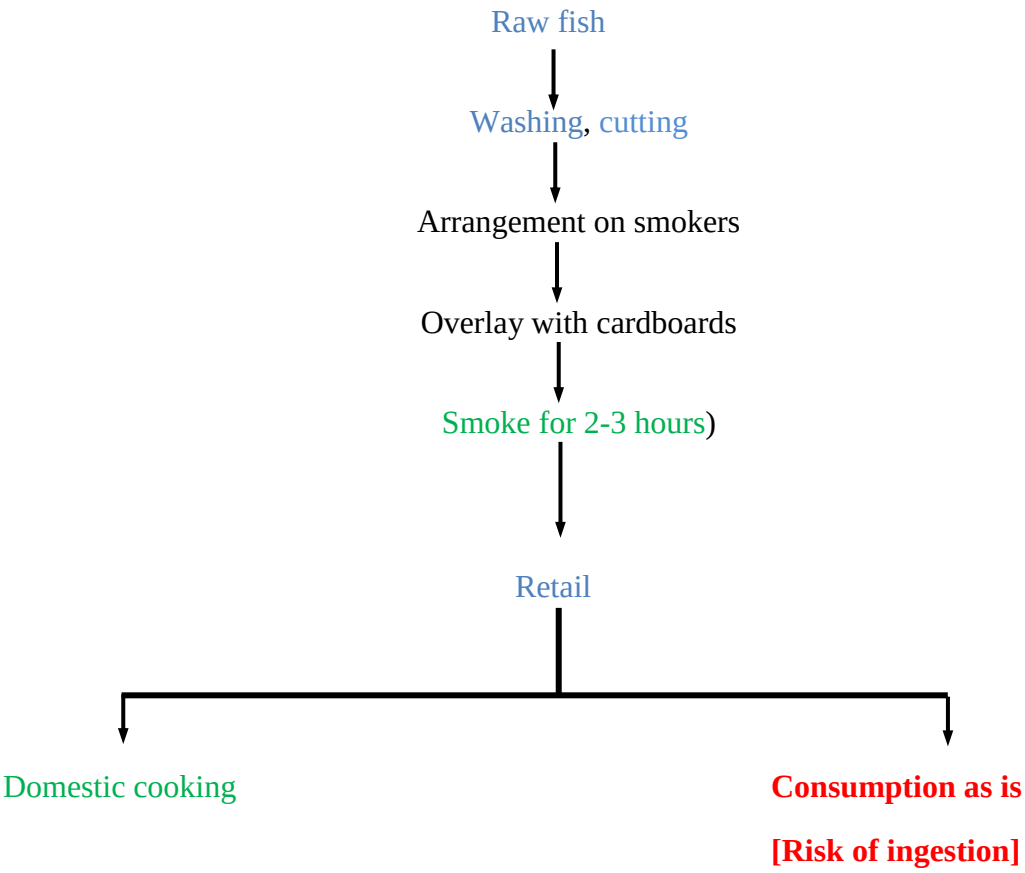


Fig. 4.25: Risk pathway for traditionally smoked fish (tuna, mackerel, herrings)

Fig. 4.26: Risk pathway for traditionally salted fish

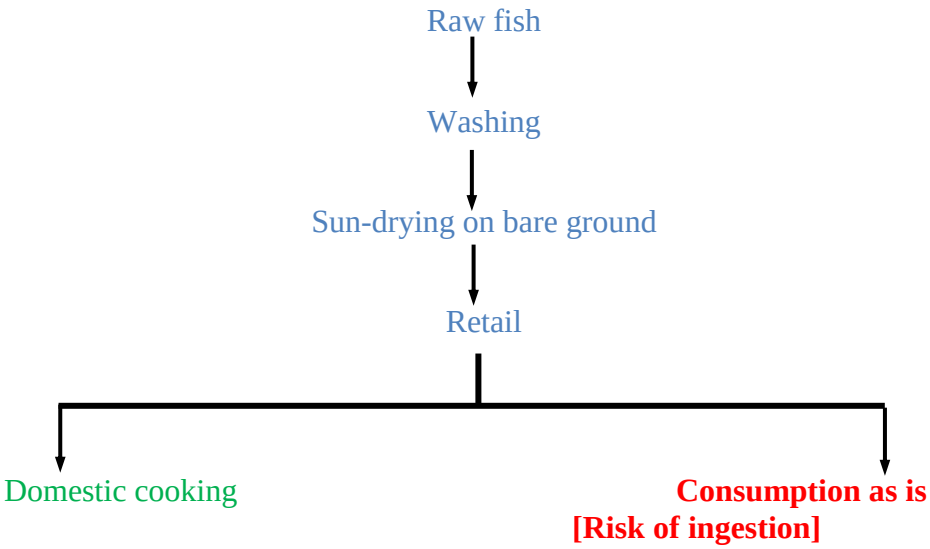
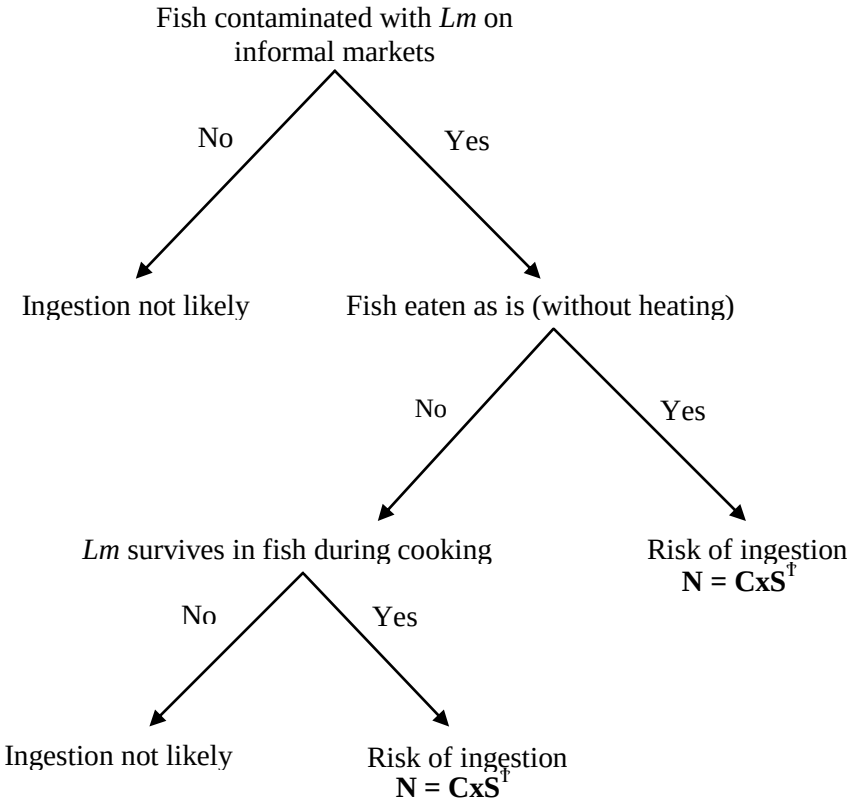


Fig. 4.27: Risk pathway for sundried fish

Following the establishment of the risk pathways, an event tree, which is a sequence of events that could result in ingestion of the hazard, was constructed for each product to enable determination of the likelihood of ingesting the hazard (Fig. 4.28 – 4.30). Event tree analysis is based on binary logic, in which an event either has or has not happened and is useful in determining the risk of ingesting *L. monocytogenes*.



†Equation 1, Section 3.7.2.3, Chapter Three

Fig. 4.28: Event tree for risk of ingestion of *Listeria monocytogenes* through consumption of traditionally smoked fish purchased from informal markets
N=number of cells C=CFU of L. monocytogenes S=Serving size of fish

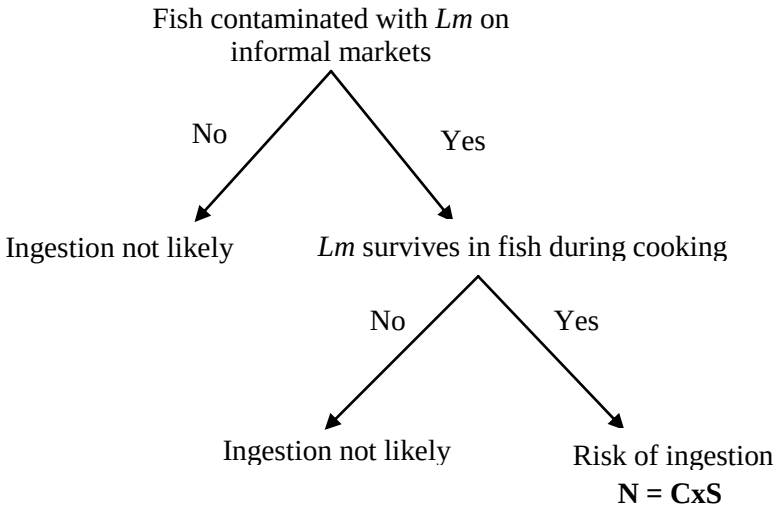


Fig. 4.29: Event tree for risk of ingestion of *Listeria monocytogenes* through consumption of salted fish purchased from informal markets

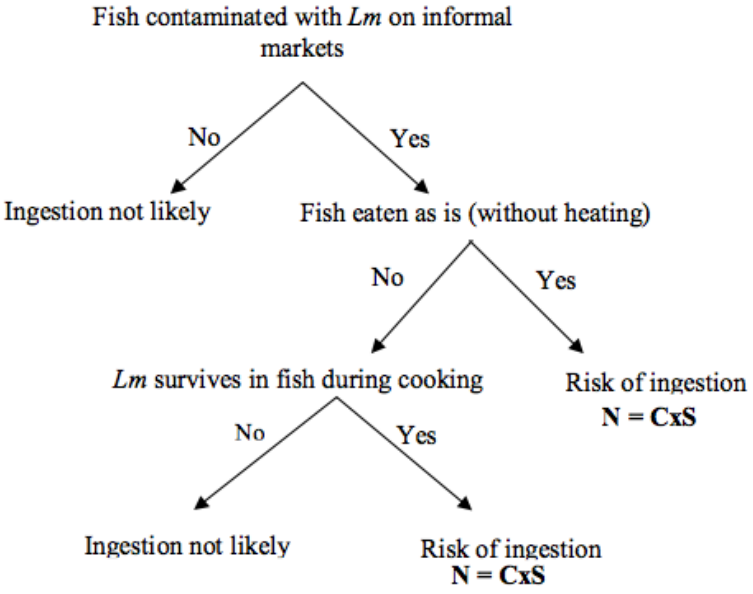


Fig. 4.30: Event tree for risk of ingestion of *Listeria monocytogenes* through consumption of sundried fish purchased from informal markets

4.10.2 Prevalence of *Listeria monocytogenes* in the fish products

As indicated in Section 3.7.2 (Chapter Three), the prevalence and concentration of *L. monocytogenes* in the fish products, as well as the likelihood of ingestion of the pathogen through the products were used as the basis for the exposure assessment.

Table 4.13 shows the number of fish samples from markets in which the pathogen was detected. The values for individual prevalence of the pathogen in each product from the respective markets are presented in Appendix 5.

Table 4.13: Average prevalence of *L. monocytogenes* in traditionally processed fish purchased from some informal markets in Accra and Tema

Product	Number of samples purchased	Number of samples positive for *L. monocytogenes*	Prevalence of *L. monocytogenes* (%)
Smoked tuna	15	12	80
Smoked mackerel	15	14	93
Smoked herrings	15	10	67
Sundried sardines	15	9	60
Koobi	15	6	40
Kako	15	8	53
Momoni	15	8	53

4.10.3 Concentration of *Listeria monocytogenes* in the fish products

The concentrations of *L. monocytogenes* in the respective traditionally processed fish have been presented in Table 4.6. The counts were generally low, ranging from 10^2 to 10^3 CFU/g.

4.10.4 Ingestion of *L. monocytogenes* through the fish products

For all products, the estimation of likely numbers of the pathogen ingested was based on the following general assumptions:

i. either traditionally processed fish is consumed as purchased from informal markets OR traditionally processed fish purchased from informal markets is not heat-treated to an extent that guarantees elimination of *L. monocytogenes* cells initially present in the product (i.e. the pathogen survives domestic cooking)

ii. all strains of *L. monocytogenes* in the fish products are virulent

For *kako* and *momoni*, an additional assumption was that the quantities reported by the respondents are actually consumed whole, although the products are used as condiments and usually break up in soups and are thus not available for direct consumption. This enabled determination of likelihood of ingestion in a worst case scenario.

From Equation 1 (Section 3.9.2.3, Chapter Three), the likely numbers of *L. monocytogenes* ingested through consumption of the respective traditionally processed fish was calculated as

$$N = C \times S \text{ ----------(1)}$$

where N = likely number of *L. monocytogenes* cells ingested
C = CFU/g of *L. monocytogenes* in the fish product
S = serving size of fish product

(Lindqvist and Westoo, 2000)

Therefore, using the average concentrations of *L. monocytogenes* in each product (Table 4.6) and the highest portion sizes of each product most often consumed at an instance

(Tables 4.9 – 4.12), the likely numbers of the pathogen ingested through consumption of each product were calculated for the communities in which the surveys were conducted (Jamestown, Tema New Town and Madina) and are presented in Tables 4.14 – 4.16**.**

Generally, in all three communities, consumers were likely to ingest more *L. monocytogenes* cells through the smoked fish products than through dried fish and salted fish (in that order). This was because the smoked fish products were consumed more frequently and in greater quantities than the dried fish and salted fish. Additionally, the concentration of *L. monocytogenes* were higher in the smoked fish products.

Table 4.14: Likely numbers of *Listeria monocytogenes* ingested through consumption of traditionally processed fish in Jamestown

	Product	Average Lm CFU/g, (C)	Quantity (g) most often consumed at an instance (S)	Likely number of Lm ingested, N=(C).(S)
Respondents	Smoked tuna	$1.20x10^3$	> 200	$> 2.40x10^5$
	Smoked mackerel	$9.00x10^2$	151-200	$1.36x10^5 – 1.80x10^5$
	Smoked herrings	$9.00x10^2$	151-200	$1.36x10^5 – 1.80x10^5$
	Sundried sardines	$2.00x10^2$	101-150	$2.02x10^4 – 3.00x10^4$
	Kako	$1.00x10^2$	5-10	$5.00x10^2 – 1.00x10^3$
	Momoni	$2.00x10^2$	5-10	$1.00x10^3 – 2.00x10^3$
	Koobi	$2.00x10^2$	41-50	$8.20x10^3 – 1.00x10^4$
Elderly	Smoked tuna	$1.20x10^3$	151-200	$1.81x10^5 – 2.40x10^5$
	Smoked mackerel	$9.00x10^2$	151-200	$1.36x10^5 – 1.80x10^5$
	Smoked herrings	$9.00x10^2$	101-150	$9.09x10^4 – 1.35x10^5$
	Sundried sardines	$2.00x10^2$	101-150	$2.02x10^4 – 3.00x10^4$
	Kako	$1.00x10^2$	5-10	$5.00x10^2 – 1.00x10^3$
	Momoni	$2.00x10^2$	5-10	$1.00x10^3 – 2.00x10^3$
	Koobi	$2.00x10^2$	21-30	$4.20x10^2 – 6.00x10^3$
Pregnant women	Smoked tuna	$1.20x10^3$	> 200	$>2.40x10^5$
	Smoked mackerel	$9.00x10^2$	151-200	$1.36x10^5 – 1.80x10^5$
	Smoked herrings	$9.00x10^2$	101-150	$9.09x10^4 – 1.35x10^5$
	Sundried sardines	$2.00x10^2$	151-200	$3.02x10^4 – 4.00x10^4$
	Kako	$1.00x10^2$	5-10	$5.00x10^2 – 1.00x10^3$
	Momoni	$2.00x10^2$	5-10	$1.00x10^3 – 2.00x10^3$
	Koobi	$2.00x10^2$	> 50	$> 1.00x10^4$
Children	Smoked tuna	$1.20x10^3$	101-150	$1.21x10^5 – 1.80x10^5$
	Smoked mackerel	$9.00x10^2$	101-150	$9.09x10^4 – 1.35x10^5$
	Smoked herrings	$9.00x10^2$	50-100	$4.50x10^4 – 9.00x10^4$
	Sundried sardines	$2.00x10^2$	50-100	$1.00x10^4 – 2.00x10^4$

Table 4.15: Likely numbers of *Listeria monocytogenes* ingested through consumption of traditionally processed fish in Tema New Town

	Product	Average Lm CFU/g, (C)	Quantity (g) most often consumed at an instance (S)	Likely number of Lm ingested, N=(C).(S)
Respondents	Smoked tuna	$2.00x10^2$	> 200	> $4.00x10^4$
	Smoked mackerel	$4.00x10^2$	151-200	$6.04x10^4$ – $8.00x10^4$
	Smoked herrings	$3.00x10^2$	151-200	$4.53x10^4$ – $6.00x10^4$
	Sundried sardines	$3.00x10^2$	101-150	$3.03x10^4$ – $4.50x10^4$
	Kako	$1.00x10^2$	5-10	$5.00x10^2$ – $1.00x10^3$
	Momoni	0	5-10	0
	Koobi	0	41-50	0
Elderly	Smoked tuna	$2.00x10^2$	151-200	$3.02x10^4$ – $4.00x10^4$
	Smoked mackerel	$4.00x10^2$	151-200	$6.04x10^4$ – $8.00x10^4$
	Smoked herrings	$3.00x10^2$	101-150	$3.03x10^4$ – $4.50x10^4$
	Sundried sardines	$3.00x10^2$	101-150	$3.03x10^4$ – $4.50x10^4$
	Kako	$1.00x10^2$	5-10	$5.00x10^2$ – $1.00x10^3$
	Momoni	0	5-10	0
	Koobi	0	21-30	0
Pregnant women	Smoked tuna	$2.00x10^2$	> 200	> $4.00x10^4$
	Smoked mackerel	$4.00x10^2$	151-200	$6.04x10^4$ – $8.00x10^4$
	Smoked herrings	$3.00x10^2$	101-150	$3.03x10^4$ – $4.50x10^4$
	Sundried sardines	$3.00x10^2$	151-200	$4.53x10^4$ – $6.00x10^4$
	Kako	$1.00x10^2$	5-10	$5.00x10^2$ – $1.00x10^3$
	Momoni	0	5-10	0
	Koobi	0	> 50	0
Children	Smoked tuna	$2.00x10^2$	101-150	$2.02x10^2$ – $3.00x10^4$
	Smoked mackerel	$4.00x10^2$	101-150	$4.04x10^4$ – $6.00x10^4$
	Smoked herrings	$3.00x10^2$	50-100	$1.50x10^4$ – $3.00x10^4$
	Sundried sardines	$3.00x10^2$	50-100	$1.50x10^4$ – $3.00x10^4$

Table 4.16: Likely numbers of *Listeria monocytogenes* ingested through consumption of traditionally processed fish in Madina

	Product	Average Lm CFU/g, (C)	Quantity (g) most often consumed at an instance (S)	Likely number of Lm ingested, N=(C).(S)
Respondents	Smoked tuna	$2.10x10^3$	> 200	> $4.20x10^5$
	Smoked mackerel	$2.90x10^3$	151-200	$4.38x10^5$ - $5.80x10^5$
	Smoked herrings	$8.00x10^2$	151-200	$1.21x10^4$ - $1.60x10^4$
	Sundried sardines	$3.00x10^2$	101-150	$3.03x10^4$ – $4.50x10^4$
	Kako	$7.00x10^2$	5-10	$3.50x10^3$ – $7.00x10^3$
	Momoni	$3.00x10^2$	5-10	$1.50x10^3$ – $3.00x10^3$
	Koobi	$6.00x10^2$	41-50	$2.50x10^4$ – $3.00x10^4$
Elderly	Smoked tuna	$2.10x10^3$	151-200	$3.20x10^5$ – $4.20x10^5$
	Smoked mackerel	$2.90x10^3$	151-200	$4.40x10^5$ – $5.80x10^5$
	Smoked herrings	$8.00x10^2$	101-150	$8.08x10^4$ – $1.20x10^5$
	Sundried sardines	$3.00x10^2$	101-150	$3.03x10^4$ – $4.50x10^4$
	Kako	$7.00x10^2$	5-10	$3.50x10^3$ – $7.00x10^3$
	Momoni	$3.00x10^2$	5-10	$1.50x10^3$ – $3.00x10^3$
	Koobi	$6.00x10^2$	21-30	$1.26x10^4$ – $1.80x10^4$
Pregnant women	Smoked tuna	$2.10x10^3$	> 200	> $4.20x10^5$
	Smoked mackerel	$2.90x10^3$	151-200	$4.40x10^5$ – $5.80x10^5$
	Smoked herrings	$8.00x10^2$	101-150	$8.08x10^4$ – $1.20x10^5$
	Sundried sardines	$3.00x10^2$	151-200	$4.53x10^4$ – $6.00x10^4$
	Kako	$7.00x10^2$	5-10	$3.50x10^3$ – $7.00x10^3$
	Momoni	$3.00x10^2$	5-10	$1.50x10^3$ – $3.00x10^3$
	Koobi	$6.00x10^2$	> 50	> $3.00x10^4$
Children	Smoked tuna	$2.10x10^3$	101-150	$2.12x10^5$ – $3.15x10^5$
	Smoked mackerel	$2.90x10^3$	101-150	$2.93x10^5$ – $4.35x10^5$
	Smoked herrings	$8.00x10^2$	50-100	$4.00x10^4$ – $8.00x10^4$
	Sundried sardines	$3.00x10^2$	50-100	$1.50x10^4$ – $3.00x10^4$

4.11 Hazard Characterization and Dose Response Assessment

The assumptions employed for the evaluation of the nature of the possible adverse health effect resulting from consumption of traditionally processed fish contaminated with *L. monocytogenes* were:

i. high risk individuals (children, elderly, pregnant women) must consume fish containing more than 10^4 CFU/g of *L. monocytogenes* to suffer listeriosis (Buchanan *et al*., 1997)

ii. low risk individuals (non-pregnant, apparently healthy individuals) must consume fish containing more than 10^9 CFU/g of *L. monocytogenes* to get ill (Schlech, 1999).

Based on the foregoing assumptions, none of the consumers (neither high risk nor low risk individuals) in all three communities surveyed was at risk of suffering listeriosis from consumption of traditionally processed fish, since the order of the count of the pathogen in the products was 10^{2-3} CFU/g in Jamestown and Madina, and 10^2 CFU/g in Tema New Town, levels less than the indicated limits of $>10^4$ CFU/g for high risk individuals and $>10^9$ CFU/g for low risk individuals.

However, since the pathogen was detected in the fish products and the products were consumed frequently in all surveyed communities, the probabilities of illness, P_{ill}, resulting from ingesting doses (N) of the pathogen in the products were calculated using the Weibull-Gamma model (Section 3.9.3, Chapter Three). The assumption employed for this calculation was that repeated exposure to the **doses** of *L. monocytogenes* in Tables 4.14 – 4.16 (i.e. 10^{3-5} cells per eating instance) *could* result in illness. Therefore, **if** those

doses could cause illness, the probabilities of occurrence of the disease would be as presented in Tables 4.17 – 4.19.

Among high risk consumers (children, the elderly and pregnant women), the probability of illness increased as the likely dose reached $\geq 10^4$ cells. The general ranges of probability of illness for this group were 10^{-4} (for dose 10^3 cells) – 0.9850 (for dose 10^5 cells) in Madina, 10^{-6} (for dose 10^2 cells) – 0.4529 (for dose 10^4 cells) in Tema New Town, and 10^{-5} (for dose 10^3 cells) – 0.9583 (for dose 10^5 cells) for Jamestown (Tables 4.17 – 4.19). This supports the assumption that doses $\geq 10^4$ CFU/g result in illness among high risk individuals (Buchanan *et al*., 1997).

For the low risk group (respondents, majority of who were aged 20-39yrs), the probabilities of illness were even lower, as expected. Values were 10^{-9} (for dose 10^3 cells) – 10^{-3} (for dose 10^5 cells) in Madina, 10^{-10} (for dose 10^2 cells) – 10^{-5} (for dose 10^4 cells) in Tema New Town, and 10^{-9} (for dose 10^3 cells) – 10^{-4} (for 10^5 cells) (Tables 4.17–4.19). This also supports the assumption that doses $\geq 10^9$ CFU/g cause illness in low risk individuals (Schlech, 1999).

Table 4.17: Probability of illness among consumers in Jamestown

Consumers	Product	Lower N	P_{ill}	Upper N	P_{ill}
Children	Smoked tuna	$1.21x10^{5}$	0.7120	$1.80x10^{5}$	0.2301
	Smoked mackerel	$9.09x10^{4}$	0.5334	$1.35x10^{5}$	0.1588
	Smoked herring	$4.50x10^{4}$	0.1766	$9.00x10^{4}$	0.0837
	Sundried sardines	$1.00x10^{4}$	0.0081	$2.00x10^{4}$	0.0041
Elderly	Smoked tuna	-	-	$2.40x10^{5}$	0.3102
	Smoked mackerel	$1.36x10^{5}$	0.7763	$1.80x10^{5}$	0.2301
	Smoked herrings	$1.36x10^{5}$	0.7763	$1.80x10^{5}$	0.2301
	Sundried sardines	$2.02x10^{4}$	0.0357	$3.00x10^{4}$	0.0097
	Kako	$5.00x10^{2}$	$1.34x10^{-5}$	$1.00x10^{3}$	$6.89x10^{-6}$
	Koobi	$8.20x10^{3}$	0.0053	$1.00x10^{4}$	0.0009
	Momoni	$1.00x10^{3}$	$5.89x10^{-5}$	$2.00x10^{3}$	$3.03x10^{-5}$
Pregnant women	Smoked tuna	-	-	$2.40x10^{5}$	0.3102
	Smoked mackerel	$1.36x10^{5}$	0.7763	$1.00x10^{5}$	0.1001
	Smoked herrings	$9.09x10^{4}$	0.5334	$1.35x10^{5}$	0.1588
	Sundried sardines	$3.02x10^{4}$	0.0814	$4.00x10^{4}$	0.0177
	Kako	$5.00x10^{2}$	$1.34x10^{-5}$	$1.00x10^{3}$	$6.89x10^{-6}$
	Koobi	-	-	$1.00x10^{4}$	0.0009
	Momoni	$1.00x10^{3}$	$5.89x10^{-5}$	$2.00x10^{3}$	$3.03x10^{-5}$
Respondents	Smoked tuna	-	-	$2.40x10^{5}$	$4.48x10^{-5}$
	Smoked mackerel	$1.36x10^{5}$	0.0001	$1.80x10^{5}$	$2.42x10^{-5}$
	Smoked herrings	$1.36x10^{5}$	0.0001	$1.80x10^{5}$	$2.42x10^{-5}$
	Sundried sardines	$2.02x10^{4}$	$1.92x10^{-6}$	$3.00x10^{4}$	$5.24x10^{-7}$
	Kako	$5.00x10^{2}$	$7.02x10^{-10}$	$1.00x10^{3}$	$3.61x10^{-10}$
	Koobi	$8.20x10^{3}$	$2.79x10^{-7}$	$1.00x10^{4}$	$4.99x10^{-8}$
	Momoni	$1.00x10^{3}$	$3.09x10^{-9}$	$2.00x10^{3}$	$1.59x10^{-9}$

Lower N, Upper N: lower and higher values of doses in Tables 4.13-4.15.
P_{ill}: probability of illness as computed from Weibull-Gamma model (Equation 2, Section 3.9.3) -: no lower value

Table 4.18: Probability of illness among high risk groups in Tema New Town

Consumers	Product	Lower N	P_{ill}	Upper N	P_{ill}
Children	Smoked tuna	$2.02x10^{2}$	$2.25x10^{-7}$	$3.00x10^{4}$	0.0097
	Smoked mackerel	$4.04x10^{4}$	0.0180	$6.00x10^{4}$	0.0397
	Smoked herring	$1.50x10^{4}$	0.0023	$3.00x10^{4}$	0.0097
	Sundried sardines	$1.50x10^{4}$	0.0023	$3.00x10^{4}$	0.0097
Elderly	Smoked tuna	$3.02x10^{4}$	0.0099	$4.00x10^{4}$	0.0177
	Smoked mackerel	$6.04x10^{4}$	0.0402	$8.00x10^{4}$	0.0680
	Smoked herrings	$3.03x10^{4}$	0.0099	$4.50x10^{4}$	0.0224
	Sundried sardines	$3.03x10^{4}$	0.0099	$4.50x10^{4}$	0.0224
	Kako	$5.00x10^{2}$	$1.56x10^{-6}$	$1.00x10^{3}$	$6.89x10^{-6}$
	Koobi	0	0	0	0
	Momoni	0	0	0	0
Pregnant women	Smoked tuna	-	-	$4.00x10^{4}$	0.0177
	Smoked mackerel	$6.04x10^{4}$	0.0402	$8.00x10^{4}$	0.0680
	Smoked herrings	$3.03x10^{3}$	$7.38x10^{-5}$	$4.50x10^{4}$	0.0224
	Sundried sardines	$4.53x10^{4}$	0.0227	$6.00x10^{4}$	0.0397
	Kako	$5.00x10^{2}$	$1.56x10^{-6}$	$1.00x10^{3}$	$6.89x10^{-6}$
	Koobi	0	0	0	0
	Momoni	0	0	0	0
Respondent	Smoked tuna	-	-	$4.00x10^{4}$	$9.69x10^{-7}$
	Smoked mackerel	$6.04x10^{4}$	$2.34x10^{-6}$	$8.00x10^{4}$	$4.27x10^{-6}$
	Smoked herrings	$4.53x10^{4}$	$1.26x10^{-6}$	$6.00x10^{4}$	$2.31x10^{-6}$
	Sundried sardines	$3.03x10^{4}$	$5.35x10^{-7}$	$4.50x10^{4}$	$1.25x10^{-6}$
	Kako	$5.00x10^{2}$	$8.20x10^{-11}$	$1.00x10^{4}$	$4.99x10^{-8}$
	Koobi	0	0	0	0
	Momoni	0	0	0	0

Lower N, Upper N: lower and higher values of doses in Tables 4.13-4.15.
P_{ill}: probability of illness as computed from Weibull-Gamma model (Equation 2, Section 3.9.3) -: no lower value

Table 4.19: Probability of illness among high risk groups in Madina

Consumers	Product	Lower N	P_{ill}	Upper N	P_{ill}
Children	Smoked tuna	$2.12x10^5$	0.2750	$3.15x10^5$	0.3877
	Smoked mackerel	$2.93x10^5$	0.3672	$4.35x10^5$	0.4754
	Smoked herring	$4.00x10^4$	0.0177	$8.00x10^4$	0.0680
	Sundried sardines	$1.50x10^4$	0.0023	$3.00x10^4$	0.0097
Elderly	Smoked tuna	$3.20x10^5$	0.3921	$4.20x10^5$	0.4662
	Smoked mackerel	$4.40x10^5$	0.4783	$5.80x10^5$	0.5462
	Smoked herrings	$8.08x10^4$	0.0693	$1.20x10^5$	0.1337
	Sundried sardines	$3.0\text{-}3x10^4$	0.0099	$4.50x10^4$	0.0224
	Kako	$3.50x10^3$	0.0001	$7.00x10^3$	0.0004
	Koobi	$1.26x10^4$	0.0016	$1.80x10^4$	0.0033
	Momoni	$1.50x10^3$	$1.64x10^{-5}$	$3.00x10^3$	$7.23x10^{-5}$
Pregnant women	Smoked tuna	-	-	$4.20x10^5$	0.4662
	Smoked mackerel	$4.40x10^5$	0.4783	$5.80x10^5$	0.5462
	Smoked herrings	$8.08x10^4$	0.0693	$1.20x10^5$	0.1337
	Sundried sardines	$4.53x10^4$	0.0227	$6.00x10^4$	0.0397
	Kako	$3.50x10^3$	0.0001	$7.00x10^3$	0.0004
	Koobi	-	-	$3.00x10^4$	0.0097
	Momoni	$1.50x10^3$	$1.64x10^{-5}$	$3.00x10^3$	$7.23x10^{-5}$
Respondent	Smoked tuna	-	-	$4.20x10^5$	0.0001
	Smoked mackerel	$4.38x10^5$	0.0002	$5.80x10^5$	0.0003
	Smoked herrings	$1.21x10^4$	$7.50x10^{-8}$	$1.60x10^4$	$1.36x10^{-7}$
	Sundried sardines	$3.03x10^4$	$5.35x10^{-7}$	$4.50x10^4$	$1.25x10^{-6}$
	Kako	$3.50x10^3$	$5.28x10^{-9}$	$7.00x10^3$	$2.33x10^{-8}$
	Koobi	$2.50x10^4$	$3.54x10^{-7}$	$3.00x10^4$	$5.24x10^{-7}$
	Momoni	$1.50x10^3$	$8.61x10^{-10}$	$3.00x10^3$	$3.79x10^{-9}$

Lower N, Upper N: lower and higher values of doses in Tables 4.13-4.15.
P_{ill}: probability of illness as computed from Weibull-Gamma model (Equation 2, Section 3.9.3). -: no lower value

Considered without regard to the communities, the probabilities of illness from ingesting the doses (N) of *L. monocytogenes* (Tables 4.14-4.15) among the different consumer groups ranged from 1 in 100 chances of illness (order of 10^{-1}) to 1 in 10^{11} chances, as summarized in Table 4.20.

Table 4.20: Summary of ranges of probability of illness among consumers (without regard to communities)

	Ranges of probability of illness			
	Low Risk Group	High Risk Group		
Product	**Respondents**	**Elderly**	**Children**	**Pregnant women**
Smoked Tuna	10^{-4} -10^{-7}	10^{-1} -10^{-3}	10^{-1} -10^{-7}	10^{-1} -10^{-2}
Smoked mackerel	10^{-3} -10^{-6}	10^{-1} -10^{-2}	10^{-1} -10^{-2}	10^{-1} -10^{-2}
Smoked herrings	10^{-6} -10^{-8}	10^{-1} -10^{-2}	10^{-1} -10^{-3}	10^{-1} -10^{-5}
Sundried sardines	10^{-6} -10^{-7}	10^{-2} -10^{-3}	*10^{-3}	*10^{-2}
Kako	10^{-8} -10^{-11}	10^{-4} -10^{-6}	-	10^{-4} -10^{-6}
Koobi	10^{-7} -10^{-8}	10^{-3} -10^{-4}	-	10^{-7} -10^{-8}
Momoni	10^{-9} -10^{-10}	10^{-5} -10^{-9}	-	10^{-5} -10^{-9}

* same order recorded for both lower dose and upper dose - not determined since data on consumption patterns for products were not collected for consumer category

4.11.1 Monte Carlo simulations

The risk estimates (probabilities of illness) presented in Tables 4.17 – 4.19 are point estimates, the use of which to determine risks is considered unsatisfactory (Lindqvist and Westoo, 2000) and has been criticized for its tendency to give errors (Cassin *et al*., 1996). Therefore, the point estimates were converted into distributions using @Risk 5.5

Software. The distributions are shown in Appendix 6**.** A few typical distributions are show in Fig. 4.31 – 4.33 as examples.

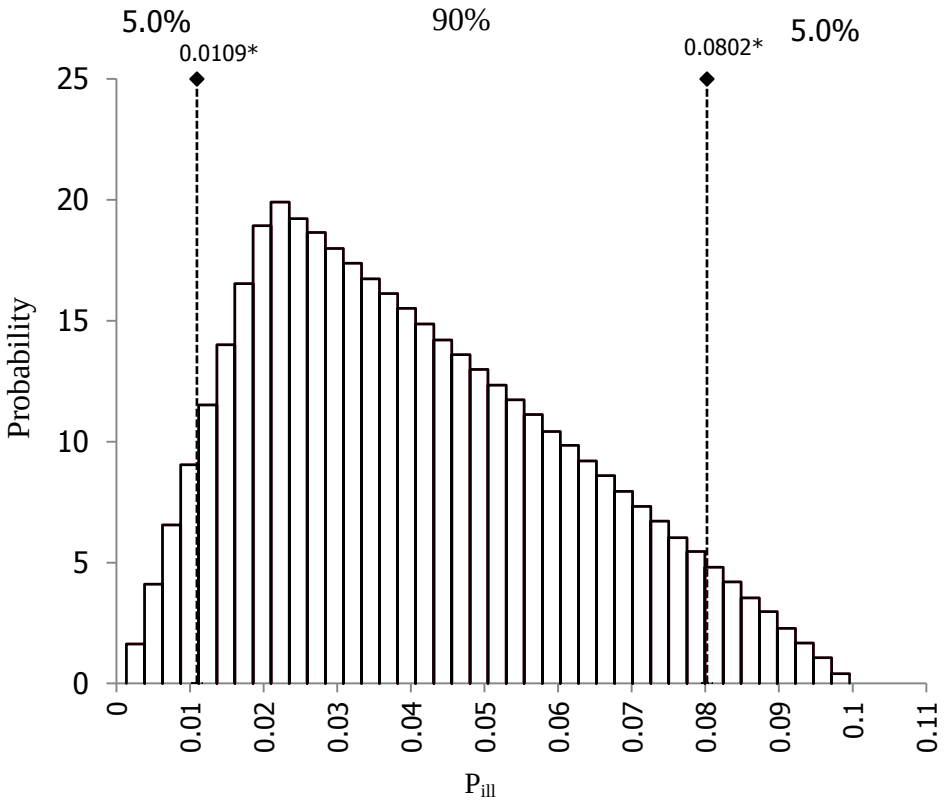


Fig. 4.31: Triangular distribution for probability of illness, P_{ill}, among the elderly in Madina consuming sundried sardines contaminated with *Listeria monocytogenes*

Fig. 4.31 shows that the minimum and maximum probabilities of illness are 0.0109 and 0.0802 (marked *), between which 90% of the probabilities of illness fell. The figure shows that about 2 in 100 chances of falling ill is the highest likelihood. Beyond this, the chances of infection diminish steadily.

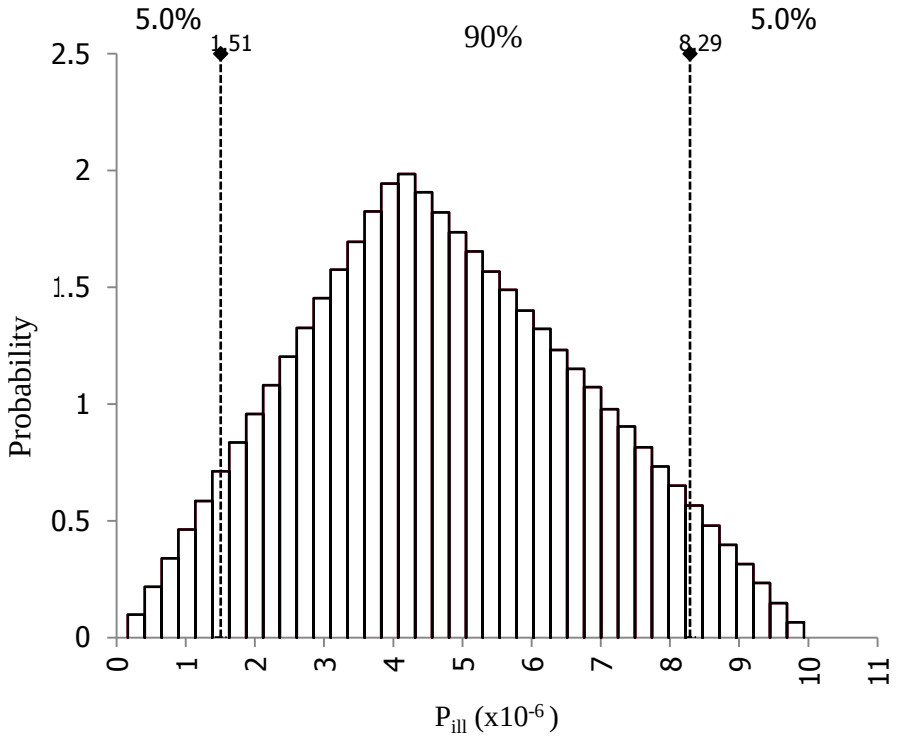


Fig. 4.32: Triangular distribution for probability of illness among respondents in Tema New Town who consumed smoked mackerel contaminated with *Listeria monocytogenes*

Among respondents in Jamestown consuming smoked mackerel, Fig. 4.32 shows that the likely tendency is an increase in the probability of infection from 2 in 1,000,000 to about 5 in 1,000,000. Beyond this range, lower probabilities of illness are expected.

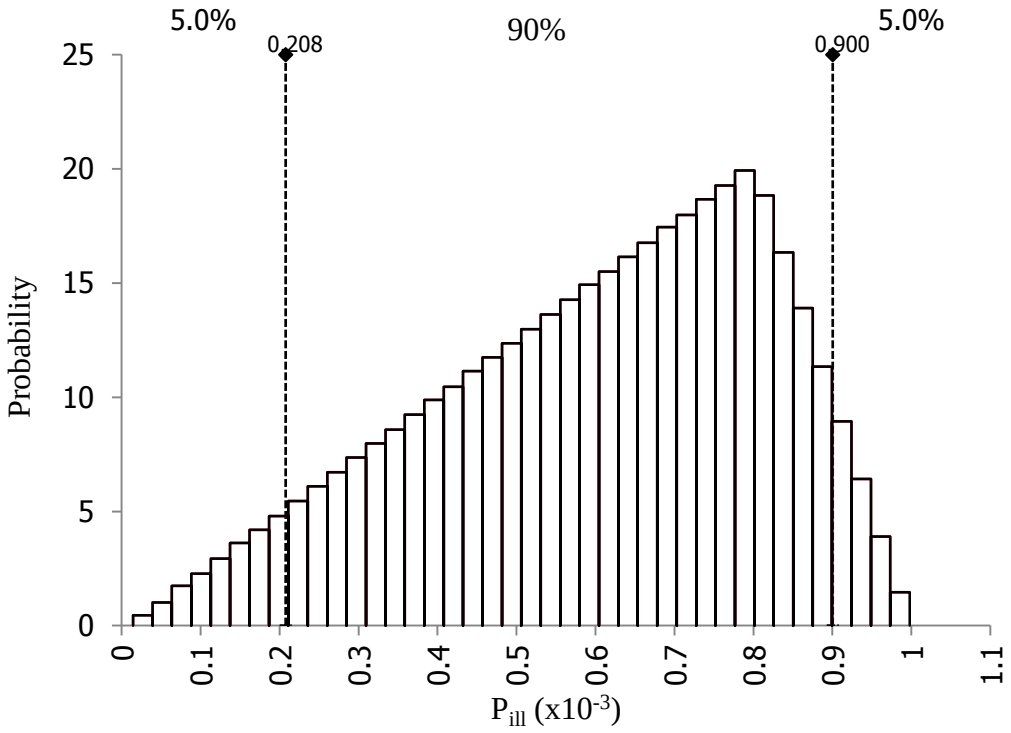


Fig. 4.33: Triangular distribution for probability of illness among pregnant women in Jamestown consuming *koobi* contaminated with *Listeria monocytogenes*

The distribution in Fig. 4.33 shows that the most likely probability of illness among pregnant women in James town as a result of consumption of *koobi* contaminated with *L. monocytogenes* is 8 in 10,000. Lower probabilities are expected beyond this level.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Traditional Fish Processing

The methods of traditional fish processing have not changed over the years. Practices during processing observed in this study were similar to those reported by earlier researchers (Essuman, 1982; Yanka, 1988; Nketstia-Tabiri, 1994; Coffie, 2002). The means by which fresh fish were transported to the processing sites made them susceptible to contamination. The use of public transport for the fish could result in contaminations with pathogens that could survive processing and pose a food safety risk to consumers.

5.1.1 Sanitation

The processing environments were generally unsanitary (Fig.4.6, 4.11 and 4.16). The processors did not conform to the acceptable conditions for processing premises (buildings, hygienic facilities and water quality programme) and general hygiene (sanitation programmes and handling practices) stipulated by the Ghana Standards Board Code of Practices (GS 235:1997).

Fish handling practices were generally poor and unhygienic among the processors. Thawing of frozen mackerel and herrings close to the ground with spillage on the same (Fig. 4.12 – 4.13), cutting of fresh ray fish on the bare ground (with only wet cardboard separating fish and soil, Fig. 4.29) and use of same bowl of water to wash several fish

(Fig. 4.14, 4.17) compromised the hygienic conditions of fish handling during traditional processing. These made contaminations very probable, and could explain the high microbial counts recorded for some of the fresh fish (Table 4.2 – 4.5).

Observations made at the processing sites also suggest that post-processing contaminations could start from the "packaging" of fish for the market. For example, in smoked fish, processors did not wash their hands before taking the finished product off the oven. While taking fish off the oven, other processors offered to help without considerations on the hygienic status of their hands or clothing (Fig. 4.10). Additionally, smoked fish on trays very placed close to the bare ground (Fig. 4.15) possibly enhanced susceptibility to post-processing microbial contamination.

In processing sundried sardines, the practice of sweeping the dried fish into heaps to be collected in baskets (Fig. 4. 19) was unsatisfactory. In addition to promoting contamination with soil microflora, physical hazards such as stones could also be introduced into the fish. This is particularly important from a food safety perspective as the fish do not go through any additional cleaning processes before being sent to the market. Similarly, the practice of stepping on salted fish (Fig. 4.27) while gathering them was not satisfactory, as contaminations could result.

5.1.2 Detection of total coliforms and *Escherichia coli* in fish during traditional processing

The results suggest that the microbial counts of fish decreased from the raw through to the processed fish (Tables 4.2 – 4.5). The absence of growth for all fresh mackerel and

herrings suggest adherence to hygienic codes of fish packaging in the frozen companies from which the fish were purchased. Oppey (2002), however, reported counts of $1.3x10^3$ CFU/g for total plate count in frozen mackerel Accra.

The microbiological quality of the water used for washing was unsatisfactory (Tables 4.2 – 4.5, Fig. 4.2 – 4.5). The total plate counts of wash water before and after use were in the order of 10^6 CFU/ml. *E. coli* and total coliform counts were also in the order of 10^3 CFU/ml. This violates the requirement that water considered ideal for food processing operations must not have any coliforms (ICMSF, 1996). Cofie (2003) recorded similar findings on the microbiological quality of water used in traditional smoking of mackerel in Accra and Tema.

The absence of microbial growth in all smoked fish (tuna, mackerel and herrings) samples collected immediately after processing suggest that the time-temperature combination of smoking was sufficient to eliminate the microorganisms. Cofie (2003) also did not record any growths for total plate count and *E. coli* in smoked fish sampled immediately after smoking. These findings emphasize the smoking stage as a critical control point in the smoked fish process flow.

In salted fish, the absence of growth for total plate count, total coliform count and *E. coli* and *L. monocytogenes* could be explained by the high concentrations of salt used (Fig. 4.30). Growths recorded after at least three (3) days sun-drying on the ground could be a result of contamination from the soil (Fig. 4.31).

In sundried sardines, the occurrence of microbial growth at every stage of processing (Table 4.4) could be due to the lack of any step in the processing chain that had the potential to eliminate microorganisms. Beyond reduction in water activity of the fish, no heating or osmotic stress strategies are employed to eliminate microorganisms. Additionally, the direct exposure of the fish to soil for days (with strong winds blowing dust over the fish), and mode of collecting fish after drying (Fig. 4.19) contributed to the generally high microbial counts obtained for this product.

5.2 General microbiology of fish on informal markets

The microbiological status of the traditionally processed fish on all the five informal markets surveyed was generally unsatisfactory. As shown in the Table 4.6, the counts were in the order of 10^6 CFU/g for total plate counts, 10^5 CFU/g for total coliforms, 10^3 CFU/g for *E. coli*, and 10^3 for *L. monocytogenes*. Contamination of foods with coliforms in general and *E. coli* in particular mostly results from unhygienic handling of foods (Jay *et al*., 2005; Hobbs and Roberts, 1987), suggesting that hygienic handling during fish sales on informal markets is unsatisfactory. Additionally, although most coliforms are not pathogenic (Prescott *et al*, 1995; Montville and Matthews, 2005) they are indicator organisms (Brock and Madigan, 1991). Therefore, an additional safety concern is that their presence in the fish products suggests the likelihood that other pathogenic microorganisms (possibly including heat-resistant strains) were also present. Other studies on the microbiological safety of fish on informal markets in Ghana reported similar findings (Adu-Gyamfi, 2006; Cofie, 2003; Oppey, 2002).

Counts obtained after processing were generally lower than those obtained for market samples. Example, whereas no counts were obtained for smoked fish sampled immediately after processing (Tables 4.2 and 4.5), counts for smoked fish on informal markets were high 10^3 - 10^6 CFU/g (Table 4.6). This suggests that post-processing handling practices either caused or contributed significantly to the contamination of the fish products. *L. monocytogenes* has the potential to contaminate fish at any point between harvesting and consumption (FAO, 1999).

5.3 Risk assessment for *Listeria monocytogenes* from the consumption of traditionally processed fish

5.3.1 Hazard identification

Listeria monocytogenes, the hazard considered in this study, causes listeriosis, a relatively rare but highly fatal disease. The pathogen is opportunistic and particularly affects segments of the population that are immunocompromised, pregnant women, unborn or newly borne infants and the elderly. Detailed information on the organism is presented in Section 2.7 (Chapter Two).

5.3.1.1 Detection of *Listeria monocytogenes* during processing

Listeria monocytogenes was not detected in fresh mackerel and herrings before washing. After washing, however**,** it was detected, suggesting that the wash water could be a source of contamination. This was confirmed when the pathogen was detected in water samples used for washing (Tables 4.2-4.5). The next unit operation after washing of the

fish was smoking. Immediately after smoking, the pathogen was no longer detectable in the smoked fish, suggesting that the heat process was adequate to eliminate *L monocytogenes* in the product.

In fresh ray fish and sardines, the occurrence of *L. monocytogenes* could be because the pre-processing operations were done close to the ground under unsanitary conditions. Additionally, contamination may have resulted from contact of the fish with soil during cutting (Fig. 4.29). In stale fish used for *momoni*, the presence of the pathogen was expected as the fish were deteriorated and heavily contaminated (Figure 4.22).

5.3.1.2 Occurrence of *Listeria monocytogenes* in traditionally processed fish on the markets

Listeria monocytogenes was detected in 53 – 80% of the fish samples obtained from the five markets in the study (Table 4.13). This was a rather high prevalence, as most studies report low values. Kwiatek (2004) found the organism in only 4% of 451 smoked fish samples, and 8% of 633 raw fish samples in Poland. In Mexico, Oroczo (2000) also recorded a prevalence of 28% in 14 samples of smoked salmon. Mahmood *et al*., (2003) detected the pathogen in 24% of 320 samples of some poultry products. The FAO (2009) also asserts that prevalence of *L. monocytogenes* in foods is generally low. Salihu *et al*. (2008) reported an incidence of 25% in 115 samples of smoked fish in Sokoto, Nigeria. The closest prevalence to the finding in the present study was the value reported by Ikeh *et al*., (2010), who detected the organism in 40% of 15 fish samples from some informal markets in Nigeria.

The absence of the pathogen in fish sampled immediately after smoking and detection in samples on the markets suggests that post-processing contamination was responsible for the occurrence. This is further supported by the observation that fish products that had antimicrobial properties such as *koobi*, *kako* and *momoni* because of their high salt concentrations had the lowest prevalences of 40%, 53% and 53%respectively. The combination of high salt content and dry nature (i.e. low water activity) probably did not support the survival of *L. monocytogenes*.

It is plausible to suggest that water activity may have had a role in the prevalence rate of *L. monocytogenes*, even though it was not determined for the products. The lower prevalence in the sundried fish (60%) than the smoked fish (67 – 80%) could be a result of the relatively lower water activity in the former. Moreover, among the smoked fish samples, the lower prevalence in herrings (67%) than tuna and mackerel (80% and 97% respectively) could be attributed to the relatively dry nature of the smoked herrings.

Detection in the dried fish could be attributed to the unsanitary practice of drying the fish on the bare ground and using brooms to sweep the products into heaps (Fig. 4.19). Contamination from soil was therefore very likely.

5.3.2 Exposure assessment

The primary objective of the exposure assessment in this study was to estimate the level of exposure of consumers to *Listeria monocytogenes* based on frequency of consumption, and the pathogen load (i.e. extent of contamination) of fish consumed. Detection of *L.*

monocytogenes in all the fish products suggests that consumers are exposed to ingestion of the pathogen if it is not eliminated before the products are consumed. Therefore, from a food safety perspective, the most important point of the processing and distribution chains of the traditionally processed fish examined in this study was the point of consumption (Fig. 4.50 and 4.51).

Some consumers indicated consuming smoked fish and sundried fish as purchased from the market, without (much) further heat processing. Such consumption practices enhance the exposure to the pathogen.

Of all the fish products, exposure through salted fish would generally be minimal as the count for samples in which the pathogen was detected was low (10^2CFU/g). Additionally, the salted fish products are generally not consumed frequently, and when used, are usually in small quantities (portion sizes of *koobi* could however be more than 50g, Appendix 3), and often heat treated.

Food service centres, both formal (registered, audited and regulated food businesses) and informal could be likely places for consumer exposure to the pathogen. However, samples obtained from food vendors showed non-detectable counts of the pathogens (Section 4.5). The absence of the pathogen in samples from food vendors even though reassuring, does not necessarily imply that cooking completely eliminates the pathogen from foods so that no risk could occur at the point of consumption. Non-detection of the pathogen could be because food vendors (as generally practiced) kept the soups on fire

throughout sales to ensure that they were hot, as cold or lukewarm food would normally not be patronized. The focus group discussions revealed that street foods stay on fire longer than in domestic cooking, where for instance light soup was cooked for approximately 45 minutes and groundnut soup for 1 hour 15 minutes. However, sometimes, street vended foods are not kept hot throughout sales. In such instances, consumers could be at risk of ingestion if the pathogen is present in the foods.

Based on the estimated cooking times of popular products such as light soup and groundnuts soup, the assumptions at Section 4.9.4 were formulated and the exposure of consumers to the pathogen determined.

5.3.2.1 Event trees

The event tree analyses (Fig. 4.28 - 4.30) were used to evaluate the consequences arising from the event of detection of *Listeria monocytogenes* leading to consumer exposure. The consequences of detection of the pathogen were followed through a series of possible paths (that may or may not eliminate the hazard) during handling.

The conditions under which consumers were at risk of ingesting *L. monocytogenes* are shown in the event trees in Figs. 4.52 – 4.55. Under those conditions, consumers were exposed to ingesting up to 10^5 cells through smoked tuna, mackerel and herrings, up to 10^4 cells through sundried sardines, and up to 10^5 cells through the salted fish products (Tables 4.14 – 4.16).

5.3.3 Hazard Characterization (dose-response assessment)

The dose response relationship was modelled using the triangular probability distribution where the 'normal' could be predicted knowing the worst and best case scenarios.

5.3.3.1 Host susceptibility

Granted that the number of cells in Tables 4.14 – 4.16 (10^3 to 10^5 cells) were consumed at an instance by the consumers, the likelihoods of suffering listeriosis (infection), P_{ill}, would be as presented in Tables 4.17 – 4.19. From the P_{ill} values in Tables 4.17 – 4.19, it is seen that the risk of infection was generally lower among the low risk groups (respondents) (P_{ill} of 10^{-4} to 10^{-11}, i.e. 1 in 10,000 to 1 in 100,000,000,000 chances of illness) than the high risk groups (10^{-1} – 10^{-6} i.e. 1 in 10 to 1 in 1,000,000 chances of illness). Although higher doses (>10^9 CFU/g, Schlech, 1999) are required to cause illness in low risk groups than in high risk groups (10^4 CFU/g, Buchanan *et al*., 1997), the results of the present study suggests that host susceptibility is a more important factor in determining probability of illness than the dose of the pathogen ingested. At equal doses, high risk groups recorded higher risk of illness (infection) than low risk individuals. For example, in Tema New Town, although the dose in smoked tuna was the same for pregnant women and respondents (10^4 cells in each case) the respective P_{ill} were 1.77×10^{-2} and 9.69×10^{-11}, about nine orders less in the low risk group.

5.3.3.2 Matrix effects

The three factors that affect the dose-response relationship include the environment (i.e. the food matrix), the pathogen (virulence characteristics), and the host (susceptibility or

immune status factors). The assumption in this work is that *L. monocytogenes* detected in the study belong to the most virulent strains. Different sub groups of the populations have been identified as least susceptible (low risk) and most

susceptible (high risk).

Among the fish products, the highest P_{ill} were recorded for the smoked fish, and the lowest for salted fish (Table 4.20). Although differences in the counts of the pathogen in the respective products were important, the key factor affecting the size of P_{ill} was the portion sizes of the products consumed at an instant. For example, although the counts of *L. monocytogenes* in sundried sardines and *koobi* were in the same order (10^2) for the elderly in Jamestown (Table 4.14), the P_{ill} value in sundried sardines was one order greater (10^{-1}) than that for *koobi* (10^{-2}) due to larger portion sizes consumed at an instance in the former.

5.3.3.3 Triangular Distributions for P_{ill}

The probability distributions (Fig. 4.55 – 4.57 and Appendix 6) show that the chances of recording a P_{ill} value beyond the most likely P_{ill} were generally low. Therefore, the chances of a larger number of consumers than recorded in Table 4.20 falling ill following ingestion of the doses of *L. monocytogenes* in Tables 4.14 – 4.16 is low. In describing the distributions of P_{ill} using the @Risk 5.5 software, the minimum values were set at one order less than the P_{ill} values for Upper N (Tables 4.17-4.19) whiles the maximum values were set at one order greater. The actual P_{ill} values or approximations of the same were used as the most likely values.

5.3.4 Risk characterization

Given that the average counts of *Listeria monocytogenes* in the traditionally processed fish were generally low (10^2 – 10^3 CFU/g), on the whole, consumers are at a low risk of ingestion. Consumers who cook the products and consume them while hot are likely to further lower their risk of ingesting the pathogen. As a result of the low counts, the number of cells that would survive cooking is expected to be less. Additionally, those that survive are expected to be injured/stressed as not to pose threats to the health of consumers. Those who consume the products as purchased from informal markets increase both their risk of ingesting the pathogen and their risk of infection.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The sanitary conditions of traditional fish processing in Accra and Tema were unsatisfactory. This could result in the occurrence of coliforms in general and *Escherichia coli* in particular (and possibly other pathogens) along some points during processing. Although the microbial counts of the products generally decreased after processing, improper post-processing handling resulted in contamination of the processed fish.

Listeria monocytogenes occurs in traditionally processed fish on informal markets, suggesting that the products could be vehicles for the transmission of the pathogen to consumers. Depending on the kind of traditionally processed fish consumed, consumers are exposed to ingesting 10^2 to 10^5 cells of *L. monocytogenes*, which could result in a 1 in 10 to 1 in 100,000,000 chances of illness. Consumers with high susceptibility to *L. monocytogenes* infection (elderly, children and pregnant women) were at a greater risk of illness than low risk individuals (non-pregnant adults aged 18 – 39 years).

Although the estimated risks of ingestion and infection were generally low, individuals who either consume traditionally processed fish purchased from informal markets as is or do not heat-treat the products sufficiently increase their risks of ingestion and infection, and vice versa.

6.2 RECOMMENDATIONS

Further studies should be conducted on

- the occurrence of *Listeria monocytogenes* in other (traditionally processed) foods with high consumption rates in Ghana to determine the exposure of consumers to those products, and estimate the associated risks of listeriosis.

- screening of placental smears for and molecular typing of *L. monocytogenes* to confirm occurrence of listeriosis. This will help to establish if the strains isolated from foods could be conclusively implicated in the occurrence of listeriosis or its symptoms.

- consumption patterns of various foods in Ghana to aid risk assessments. Information on portion sizes and frequency of consumption of foods are essential for determining the exposure of consumers to food-borne hazards. Without this information, a comprehensive risk assessment cannot be conducted.

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APPENDICES

Appendix 1: Consumer Questionnaire

DEPARTMENT OF NUTRITION AND FOOD SCIENCE
UNIVERSITY OF GHANA, LEGON

RISK ASSESSMENT FOR *LISTERIA MONOCYTOGENES* IN TRADITIONALLY PROCESSED FISH IN GHANA

Dear respondent, this questionnaire seeks to solicit some information on the consumption of traditionally processed fish in Ghana, as part of an MPhil Food Science Thesis on the topic above. The information you provide in this document will be treated as confidential and used for academic purposes only. Thank you.

Date: _____

Area: _____

Kindly tick (√) the responses that apply to you. Where appropriate, write out your own responses in the spaces provided.

A: BACKGROUND INFORMATION

RESPONSE

[For interviewer use only]

1. Sex: 1=Male 2=Female ☐

2. Age:
   1=Less than 20 years
   2=20 – 29 years
   3=30 – 39 years
   4=40 – 49 years
   5=50 years and above ☐

3. Highest level of education
   1=None 4=Secondary
   2=Primary 5=Tertiary
   3=Middle School/JHS 6=Other, specify..... ☐

B: FISH CONSUMPTION PATTERNS

4. Do you consume traditionally processed fish? ☐
   1 = Yes
   2 = No

5. If yes to 4, which of the following traditionally processed fish do you consume?

1=Smoked fish 3=Smoke-dried fish
2=Salted fish 4=Fried fish
5=Other, specify.....

6. Which species of traditionally processed fish do you consume?

1 = Mackerel
2 = Tilapia
3 = Herrings
4 = Other, specify.....

How often do you consume the fish products?

7. Smoked tuna

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify.....

8. Smoked mackerel

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify.....

9. Smoked herrings

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify.....

10. *Koobi*

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify.....

11. *Kako*

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify.....

12. *Momoni*

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify..... ☐

13. Sundried sardines

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify..... ☐

How much do you consume at an instance?

14. Smoked tuna

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g ☐

15. Smoked mackerel

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g ☐

16. Smoked herrings

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g ☐

17. *Koobi*

1= 10-20g
2= 21-30g ☐
3= 31-40g
4= 41-50g
5= >50g

18. *Kako*

1= 5-10g 4= 31-40g
2= 11-20g 5= >40g ☐
3= 21-30g

19. *Momoni*

1= 5-10g
2= 11-20g
3= 21-30g
4 = 31-30g
5 = > 50g

20. Sundried sardines

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g

C: CONSUMPTION BY HIGH RISK GROUPS

I. CHILDREN

21. Do you have children (6mo to 6years) in your home?

1 = Yes 2 = No

If ***No*** to Q21, go to **Q29**

How often do they consume traditionally processed fish?

22. Smoked tuna

1=Daily
2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify.....

23. Smoked mackerel

1=Daily
2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify.....

24. Smoked herrings

1=Daily
2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify.....

How much do they consume at an instance?

25. Smoked tuna
    1=50-100g
    2=101-150g
    3=151 -200g
    4=More than 200g ☐

26. Smoked mackerel
    1=50-100g
    2=101-150g
    3=151 -200g
    4=More than 200g ☐

27. Smoked herrings
    1=50-100g
    2=101-150g
    3=151 -200g
    4=More than 200g ☐

28. Sundried sardines
    1=50-100g
    2=101-150g
    3=151 -200g
    4=More than 200g ☐

II. THE ELDERLY

29. Do you have elderly people (≥60y) in your home>
    1 = Yes 2 = No ☐

If ***No*** to Q29, go to **Q 37**

How often do they consume traditionally processed fish?

30. Smoked tuna
    1=Daily 2=Once a month
    3=2 – 3 times a week
    4=Once a week
    5=Other, specify..... ☐

31. Smoked mackerel

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify.....

32. Smoked herrings

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify.....

How much do they consume at an instance?

33. Smoked tuna

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g

34. Smoked mackerel

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g

35. Smoked herrings

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g

36. Sundried sardines

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g

III. PREGNANT WOMEN

37. Do you have a pregnant woman/women in your home?

1 = Yes 2 = No

If No to **Q 37**, go to **Q 45**

How often do they consume traditionally processed fish?

38. Smoked tuna

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify..... ☐

39. Smoked mackerel

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify..... ☐

40. Smoked herrings

1=Daily 2=Once a month
3=2 – 3 times a week
4=Once a week
5=Other, specify..... ☐

How much do they consume at an instance?

41. Smoked tuna

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g ☐

42. Smoked mackerel

1=50-100g 4 = More than 200g
2=101-150g
3=151 -200g ☐

43. Smoked herrings

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g ☐

44. Sundried sardines

1=50-100g
2=101-150g
3=151 -200g
4=More than 200g

D: FISH HANDLING PRACTICES

45. Where do you buy your traditionally processed fish?

1 = Informal market
2 = Processing sites
3 = House to house vendors
4 = Other, specify.....

46. How much traditionally processed fish do you often purchase?

1 = Enough quantity for one meal
2 = Enough quantity for three days
3 = Enough quantity for one week
4 = Enough quantity for one month

47. How do you store unused purchased traditionally processed fish?

1 = In a refrigerator
2 = Under the sun during the day, and room temperature at dusk
3 = In polythene bags at room temperature
4 = Other, specify

48. In what form(s) do you consume traditionally processed fish?

1 = Consumed as is
2 = Cooked in soups
3 = Cooked in stews
4 = Roasted
5 = Washed and broken into fresh pepper sauce

THANK YOU

Appendix 2: Processor Questionnaire

DEPARTMENT OF NUTRITION AND FOOD SCIENCE
UNIVERSITY OF GHANA, LEGON

RISK ASSESSMENT FOR *LISTERIA MONOCYTOGENES* IN TRADITIONALLY PROCESSED FISH IN GHANA

Dear respondent, this questionnaire seeks to solicit some information on traditional fish processing in Ghana, as part of an MPhil Food Science Thesis on the topic above. The information you provide in this document will be treated as confidential and used for academic purposes only. Thank you.

Date: _____

Area: _____

Processor Code:_____

Kindly tick (√) the responses that apply to you. Where appropriate, write out your own responses in the spaces provided.

A: BACKGROUND INFORMATION

RESPONSE

[For interviewer use only]

49. Sex: 1=Male 2=Female ☐

50. Age:
1=Less than 20 years
2=20 – 29 years
3=30 – 39 years
4=40 – 49 years
5=50 years and above ☐

51. Highest level of education received
1=None
2=Primary
3=Middle School/JHS
4=Secondary
5=Tertiary
6=Other, specify..... ☐

52. How long have you been in the fish processing business?
1= 1-5 years
2= 6-10 years
3= 11-15 years
4= 16-20 years
5= More than 20 years ☐

53. What kind of fish products do you process? Tick as many as apply to you.

1=Smoked fish
2=Salted Fish
3=Dried fish
4=All the above
5=Other, specify.....

B: RAW MATERIAL ACQUISITION

54. What kind of fish do you process?

1 = Marine fish 2 = Freshwater fish

55. Where do you get your raw fish from?

1 = Fishermen
2 = Fishmongers
3 = Cold Store
3 = Open market
4 = Other, specify.....

56. What species of fish do you process?

1=Salmon
2=Tuna
3=Tilapia
4=Other, specify.....

57. Do you inspect fresh fish before purchasing?

1=Yes 2=No

58. If yes to 9, what do you look out for?

1=Colour of eyes
2=Colour of gills
3=Skin surface (smooth or slimy)
4=Other, specify.....

D: TRANSPORTATION OF RAW FISH

59. How long does it take to transport raw fish to the processing site?

1=Less than 30 minutes
2=30mins – 1 hour
3=More than 1 hour, less than 10 hours
4=More than 10 hours, less than 24 hours
5= More than 24 hours

60. How do you transport the raw fish to the processing site?

1=By foot
2=Public transport
3=Private transport
4=Refrigerated truck/van
5=Other, specify.....

61. What containers do you use to carry the raw fish during transportation?

1=Basket
2=Basin
3=Ice chest
4=Other, specify

E: PROCESSING OF FISH

62. Do you wash your hands before starting processing?

1=Yes 2=No

63. What do you use to wash your hands?

1= Only water 2=Water and soap
3=Other, specify.....

64. How long do you keep the fish before starting processing?

1=Less than 30 minutes
2=30mins – 1 hour
3=More than 1hour, less than 1 day
4=More than 1 day, less than 1 week?

65. How do you keep raw fish before starting processing?

1=At room temperature
2=In a fridge
3=In a freezer
4=Other, specify.....

66. Describe how you process your fish.

DESCRIPTION OF PROCESSING METHODS

(Space for interviewer use only)

67. How do you know when raw fish is adequately processed?

68. How much fish do you process at a time/what constitutes a batch?
    1= Less than 1 carton
    2= 1 – 5 cartons
    3= 6 – 10 cartons
    4= More than 10 cartons

69. What do you do to keep raw fish from spoiling when processing is delayed?

F: HANDLING AND STORAGE OF PROCESSED FISH

70. Where do you store processed fish?
    1= Regular room

2= Wooden shed
3= In refrigerator
4= In deep freezer, freezer compartments of refrigerators
4= Other, specify.....

71. How are the processed fish stored?
1 = In basket/sacks
2 = In perforated boxes
3 = In solid boxes (not perforated)
4 = Arranged on wooded trays
5 = Other, specify.....

72. For how long after processing do you store fish before selling?
1= Less than 1 day
2= 1 – 3 days
3= More than 3 days, less than 1 week
4= 1 week – 1 month
5 = More than a month

G. TRANSPORTATION OF PROCESSED FISH

73. Approximately how long does it take to transport processed fish from the storage/processing site to the market?
1= Less than 30 minutes
2= 30 mins – 2 hours
3= 3 – 6 hours
4= 4 – 12 h
5= More than 12 hours

74. How do you transport processed fish to the market?
1= By foot
2= Public transport
3= Private transport
4= Refrigerated truck/van
5= Other, specify.....

75. Which markets do you send your processed fish to?

THANK YOU

Appendix 3: Responses from Consumer Survey

I. Data on Respondents

Table 1: Gender distribution of respondents

	Jamestown		Tema New Town		Madina	
Gender	Number	%	Number	%	Number	%
Male	55	36.7	49	32.7	34	22.7
Female	95	63.3	101	67.3	116	77.3
Total	150	100	150	100	150	100

Table 2: Age distribution of respondents

	Jamestown		Tema New Town		Madina	
Age group (yrs)	Number	%	Number	%	Number	%
Less than 20	9	6	2	1.3	6	4
20-29	59	39.3	66	44	41	27.3
30-39	49	32.7	44	29.3	53	35.3
40-49	20	13.3	16	10.7	19	12.7
50 and above	13	8.7	22	14.7	31	20.7
Total	150	100	150	100	150	100

Table 3: Highest level of education attained by respondents

	Jamestown		Tema New Town		Madina	
Level	Number	%	Number	%	Number	%
None	7	4.7	8	5.3	5	3.3
Primary	40	26.7	46	30.7	35	23.3
Middle School/JHS	52	34.7	59	39.3	45	30
Secondary	51	34	37	24.7	53	35.3
Tertiary	0	0	0	0	12	8
Total	150	100	150	100	150	100

Table 4: Frequency of tuna consumption among respondents

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	38	25.3	22	14.7	40	26.7
2-3 times a week	105	70	114	76	70	46.7
Once a week	7	4.7	14	9.3	40	26.7
Total	150	100	150	100	150	100

Table 5: Quantities of smoked tuna often consumed at an instance by respondents

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
101-150	9	6	10	6.7	13	8.7
151-200	46	30.7	63	42	32	21.3
More than 200	95	63.3	77	51.3	105	70
Total	150	100	150	100	150	100

Table 6: Frequency of consumption of smoked salmon among respondents

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	Percentage	Number	%
Daily	50	33.3	48	32	72	48
2-3 times a week	90	60	93	62	67	44.7
Once a week	10	6.7	9	6	11	7.3
Total	150	100	150	100	150	100

Table 7: Quantities of smoked salmon often consumed at an instance by respondents

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
101-150	16	10.7	20	13.3	12	8
151-200	60	40	62	41.3	67	44.7
More than 200	74	49.3	68	45.3	71	47.3
Total	150	100	150	100	150	100

Table 8: Frequency of consumption of smoked herring among respondents

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	7	4.7	1	0.7	0	0
2-3 times a week	40	26.7	53	35.3	0	0
Once a week	76	50.7	77	51.3	55	36.7
Once a month	27	18	19	12.7	95	63.3
Total	150	100	150	100	150	100

Table 9: Quantities of smoked herring often consumed at an instance by respondents

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
50-100	13	8.7	16	10.7	29	19.3
101-150	39	26	65	43.3	87	58
151-200	88	58.7	61	40.7	34	22.7
More than 200	10	6.7	8	5.3	0	0
Total	150	100	150	100	150	100

Table 10: Frequency of consumption of momoni among respondents

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	0	0	0	0	0	0
2-3 times a week	6	4	3	2	0	0
Once a week	73	48.7	86	57.3	34	22.7
Once a month	24	16	25	16.7	48	32
Never	47	31.3	36	24	68	45.3
Total	150	100	150	100	150	100

Table 11: Quantities of momoni often consumed at an instance by respondents

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
5-10g	53	35.3	48	32	55	36.7
11-20	41	27.3	52	34.7	27	18
21-30	9	6	14	9.3	0	0
Not applicable	47	31.3	36	24	68	45.3
Total	150	100	150	100	150	100

Table 12: Frequency of koobi consumption among respondents

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	0	0	0	0	0	0
2-3 times a week	0	0	0	0	0	0
Once a week	48	32	63	42	10	6.7
Once a month	98	65.3	81	54	95	63.3
Never	4	2.7	6	4	45	30
Not applicable	0	0	0	0	0	0
Total	150	100	150	100	150	100

Table 13: Quantities of koobi often consumed at an instance by respondents

	Jamestown		Tema New Town		Madina	
Quantities (g)	Number	%	Number	%	Number	%
10-20	9	6	14	9.3	5	3.3
21-30	44	29.3	24	16	24	16
31-40	10	6.7	17	11.3	11	7.3
41-50	66	44	56	37.3	38	25.3
More than 50	17	11.3	33	22	27	18
Not applicable	4	2.7	6	4	45	30
Total	150	100	150	100	150	100

Table 14: Frequency of consumption of kako among respondents

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	0	0	0	0	0	0
2-3 times a week	19	12.7	16	10.7	0	0
Once a week	43	28.7	46	30.7	15	10
Once a month	46	30.7	48	32	70	46.7
Never	42	28	40	26.7	65	43.3
Not applicable	0	0	0	0	0	0
Total	150	100	150	100	150	100

Table 15: Quantities of kako often consumed at an instance by respondents

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
5-10g	58	38.7	60	40	43	28.7
11-20	30	20	26	17.3	29	19.3
21-30	20	13.3	24	16	13	8.7
Not applicable	42	28	40	26.7	65	43.3
Total	150	100	150	100	150	100

Table 16: Frequency of consumption of sundried sardines among consumers

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number of respondents	%	Number	%
Daily	0	0	0	0	0	0
2-3 times a week	17	11.3	24	16	0	0
Once a week	34	22.7	46	30.7	9	6
Once a month	99	66	80	53.3	124	82.7
Never	0	0	0	0	17	11.3
Not applicable	0	0	0	0	0	0
Total	150	100	150	100	150	100

Table 17: Quantities of sundried sardines often consumed at an instance by consumers

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
50-100	35	23.3	41	27.3	49	32.7
101-150	60	40	55	36.7	57	38
151-200	55	36.7	54	36	27	18
More than 200	0	0	0	0	17	11.3
Total	150	100	150	100	150	100

Table 18: Purchasing sites for traditionally processed fish

	Jamestown		Tema New Town		Madina	
	Number	%	Number	%	Number	%
Processing Site	150	100	73	48.7	0	0
Informal market	91	60.7	150	100	150	100
House to House Vendors	146	97.3	149	99.3	136	90.7

Table 19: Quantities of traditionally processed fish often purchased at an instance

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
Enough for one meal	132	88	147	98	148	98.7
Enough for three days	18	12	3	2	71	0
Enough for a week	0	0	0	0	0	

Table 20: Use of traditionally processed fish among respondents

	Jamestown		Tema New Town		Madina	
How used	Number	%	Number	%	Number	%
Used in stews	150	100	150	100	150	100
Used in soups	150	100	150	100	150	100
Broken into fresh, unheated pepper sauce	146	97.3	133	88.7	52	34.7

II. Data on Children (6months to 6years old)

Table 21: Respondents with children aged 6months to 6years in their household

	Jamestown		Tema New Town		Madina	
	Number	%	Number	%	Number	%
Yes	97	64.7	97	64.7	113	75.3
No	53	35.3	53	35.3	37	24.6
Total	150	100	150	100	150	100

Table 22: Frequency of consumption of smoked tuna among children

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	10	6.7	10	6.7	30	20
2-3 times a week	74	49.3	13	8.7	50	33.33
Once a week	13	8.7	74	49.3	33	22
Not applicable	53	35.3	53	35.3	37	24.6
Total	150	100	150	100	150	100

Table 23: Quantities of smoked tuna often consumed at an instance by children

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
50-100	26	17.3	26	17.3	75	50
101-150	71	47.3	71	47.3	38	25.3
Not applicable	53	35.3	53	35.3	37	24.6
Total	150	100	150	100	150	100

Table 24: Frequency of consumption of smoked salmon among children

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	32	21.3	32	21.3	50	33.3
2-3 times a week	64	42.7	64	42.7	53	35.3
Once a week	1	0.7	1	0.7	10	6.7
Not applicable	53	35.3	53	35.3	37	24.7
Total	150	100	150	100	150	100

Table 25: Quantities of smoked salmon often consumed at an instance by children

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
50-100	29	19.3	29	19.3	62	41.3
101-150	62	41.3	62	41.3	48	32
151-200	6	4	6	4	3	2
Not applicable	53	35.3	53	35.3	37	24.7
Total	150	100	150	100	150	100

Table 26: Frequency of consumption of smoked herring among children

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
2-3 times a week	9	6	9	6	39	26
Once a week	74	49.3	74	49.3	62	41.3
Once a month	14	9.3	14	9.3	37	24.7
Not applicable	53	35.3	53	35.3	12	8
Total	150	100	150	100	150	100

Table 27: Quantities of smoked herring often consumed at an instance by children

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
50-100	72	48	72	48	80	53.3
101-150	25	16.7	25	16.7	21	14
Not applicable	53	35.3	53	35.3	49	32.7
Total	150	100	150	100	150	100

Table 28: Frequency of consumption of sundried sardines among children

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Once a week	15	10	15	10	2	1.3
Once a month	82	54.7	82	54.7	87	58.7
Never	0	0	0	0	37	24
Not applicable	53	35.3	53	35.3	24	16
Total	150	100	150	100	150	100

Table 29: Quantities of sundried sardines often consumed at an instance by children

	Jamestown		Tema New Town		Madina	
Quantity (g)	Frequency	%	Frequency	%	Number	%
50-100g	97	64.7	97	64.7	86	58.7
101-150g	0	0	0	0	3	1.3
Not applicable	53	35.3	53	35.3	61	40
Total	150	100	150	100	150	100

III. Data on the Elderly (≥60years old)

Table 30: Respondents in James Town with the elderly (≥60years old) in their households

	Jamestown		Tema New Town		Madina	
	Number	%	Number	%	Number	%
Yes	104	69	93	62	80	53
No	46	31	57	38	70	47
Total	150	100	150	100	150	100

Table 31: Frequency of consumption of smoked tuna among the elderly

	Jamestown		Tema New Town		Madina	
	Number	%	Number	%	Number	%
Daily	54.0	36.0	40.0	26.7	2.0	1.3
2-3 times a week	48.0	32.0	45.0	30.0	25.0	16.7
Once a week	2.0	1.3	8.0	5.3	53.0	35.3
Not applicable	46.0	30.7	57.0	38.0	70.0	46.7
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 32: Quantities of smoked tuna often consumed at an instance by the elderly

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
101-150	35.0	23.3	25.0	16.7	18.0	12.0
151-200	37.0	24.7	39.0	26.0	37.0	24.7
More than 200	32.0	21.3	29.0	19.3	25.0	16.7
Not applicable	46.0	30.7	57.0	38.0	70.0	46.7
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 33: Frequency of consumption of smoked salmon among the elderly

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	31.0	20.7	24.0	16.0	12.0	8.0
2-3 times a week	54.0	36.0	55.0	36.7	57.0	38.0
Once a week	19.0	12.7	14.0	9.3	11.0	7.3
Not applicable	46.0	30.7	57.0	38.0	70.0	46.7
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 34: Quantities of smoked salmon often consumed at an instance by the elderly

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
101-150	29.0	19.3	29.0	19.3	21.0	14.0
151-200	72.0	48.0	54.0	36.0	54.0	36.0
More than 200	3.0	2.0	10.0	6.7	5.0	3.3
Not applicable	46.0	30.7	57.0	38.0	70.0	46.7
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 35: Frequency of consumption of smoked herrings among the elderly

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	3.0	2.0	18.0	12.0	2.0	1.3
2-3 times a week	18.0	12.0	23.0	15.3	6.0	4.0
Once a week	70.0	46.7	46.0	30.7	51.0	34.0
Once a month	13.0	8.7	6.0	4.0	21.0	14.0
Not applicable	46.0	30.7	57.0	38.0	70.0	46.7
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 36: Quantities of smoked herring often consumed at an instance by the elderly

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
50-100	19.0	12.7	13.0	8.7	31.0	20.7
101-150	46.0	30.7	41.0	27.3	38.0	25.3
151-200	39.0	26.0	39.0	26.0	11.0	7.3
Not applicable	46.0	30.7	57.0	38.0	70.0	46.7
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 37: Frequency of consumption of momoni among the elderly

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Once a week	5.0	3.3	2.0	1.3	3.0	2.0
Once a month	30.0	20.0	31.0	20.7	18.0	12.0
Never	69.0	46.0	60.0	40.0	59.0	38.0
Not applicable	46.0	30.7	57.0	38.0	70.0	48.0
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 38: Quantities of momoni often consumed at an instance by the elderly

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
5-10	18.0	12.0	17.0	11.3	12.0	8.0
11-20	17.0	11.3	16.0	10.7	9.0	6.0
Not applicable	115.0	76.7	117.0	78.0	129.0	86.0
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 39: Frequency of consumption of kako among the elderly

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Once a month	33.0	22.0	32.0	21.3	18.0	12.0
Never	71.0	47.3	61.0	40.7	62.0	41.3
Not applicable	46.0	30.7	57.0	38.0	70.0	46.7
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 40: Quantity of kako often consumed at an instance by the elderly

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
5-10	16.0	10.7	16.0	10.7	8.0	5.3
11-15	17.0	11.3	16.0	10.7	10.0	6.7
Not applicable	117.0	78.0	118.0	78.7	132.0	88.0
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 41: Frequency of consumption of koobi among the elderly

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Once a month	38.0	25.3	32.0	21.3	33.0	22.0
Never	66.0	44.0	61.0	40.7	47.0	31.3
Not applicable	46.0	30.7	57.0	38.0	70.0	46.7
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 42: Quantities of koobi often consumed at an instance by the elderly

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
10-20	3.0	2.0	1.0	0.7	3.0	2.0
21-30	23.0	15.3	20.0	13.3	19.0	12.7
31-40	11.0	7.3	9.0	6.0	10.0	6.7
41-50	1.0	0.7	2.0	1.3	1.0	0.7
Not applicable	112.0	74.7	118.0	78.7	117.0	78.0
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 43: Frequency of consumption of sundried sardines among the elderly

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
2-3 times a week	30.0	20.0	30.0	20.0	0.0	0.0
Once a week	29.0	19.3	38.0	25.3	39.0	26.7
Once a month	45.0	30.0	25.0	16.7	41.0	27.3
Not applicable	46.0	30.7	57.0	38.0	70.0	46.0
Total	150.0	100.0	150.0	100.0	150.0	100.0

Table 44: Quantities of sundried sardines often consumed at an instance by the elderly

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
50-100	23.0	15.3	15.0	10.0	36.0	24.0
101-150	51.0	34.0	53.0	35.3	37.0	24.7
151-200	30.0	20.0	25.0	16.7	7.0	5.3
Not applicable	46.0	30.7	57.0	38.0	70.0	46.0
Total	150.0	100.0	150.0	100.0	150.0	100.0

IV. Data on Pregnant Women

Table 45: Respondents with pregnant women in their household

	Jamestown		Tema New Town		Madina	
	Number	%	Number	%	Number	%
Yes	58	38.7	50	33.3	46	30.67
No	92	61.3	100	66.7	104	69.33
Total	150	100	150	100	150	100

Table 46: Frequency of consumption of smoked tuna among pregnant women

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	34	22.7	16	10.7	4.67	4.67
2-3 times a week	24	16	34	22.7	23.33	23.33
Once a week	0	0	0	0	0.00	0.00
Once a month	0	0	0	0	2.67	2.67
Not applicable	92	61.3	100	66.7	69.33	69.33
Total	150	100	150	100	100	100

Table 46: Quantities of smoked tuna often consumed at an instance by pregnant women

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
101-150	0	0	2	1.3	1	0.67
151-200	12	8	18	12	9	6
More than 200	46	30.7	30	20	36	24
Not applicable	92	61.3	100	66.7	104	69.3
Total	150	100	150	100	150	100

Table 48: Frequency of consumption of smoked salmon among pregnant women

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Daily	23	15.3	14	9.3	7	4.67
2-3 times a week	35	23.33	36	24	33	22
Once a week	0	0	0	0	0	0
Once a month	0	0	0	0	6	4
Not applicable	92	61.33	100	66.7	104	69.33
Total	150	100	150	100	150	100

Table 49: Quantities of smoked salmon often consumed at an instance by pregnant women

	Jamestown		Tema New		Madina	
Quantity (g)	Number	%	Number	%	Number	%
101-150	10	6.7	4	2.7	2	1.33
151-200	19	12.7	19	12.7	22	14.67
More than 200	29	19.3	27	18	22	14.67
Not applicable	92	61.3	100	66.7	104	69.33
Total	150	100	150	100	150	100

Table 50: Frequency of consumption of smoked herring among pregnant women

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
2-3 times a week	24	16	22	14.7	0	0
Once a week	34	22.7	28	18.7	46	30.67
Not applicable	92	61.3	100	66.7	104	69.33
Total	150	100	150	100	150	100

Table 51: Quantities of smoked herring often consumed at an instance by pregnant women

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
50-100	7	4.7	4	2.7	9	6
101-150	28	18.7	26	17.3	17	11.33
151-200	22	14.7	18	12	19	12.67
More than 200	1	0.7	2	1.3	1	0.67
Not applicable	92	61.3	100	66.7	104	69.33
Total	150	100	150	100	150	100

Table 52: Frequency of consumption of momoni among pregnant women

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Once a week	54	36	48	32	23	15.33
Once a month	0	0	0	0	17	11.33
Never	4	2.7	2	1.3	104	69.33
Not applicable	92	61.3	100	66.7	6	4
Total	150	100	150	100	150	100

Table 53: Quantities of momoni often consumed at an instance by pregnant women

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
5-10g	21	14	21	14	27	18
11-20	17	11.3	21	14	12	8
21-30	16	10.7	6	4	1	0.67
31-40	0	0	102	68	0	0
Not applicable	96	64	0	0	110	73.33
Total	150	100	150	100	150	100

Table 54: Frequency of consumption of kako among pregnant women

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Once a week	40	26.7	37	24.7	12	8
Once a month	10	6.7	8	5.3	22	14.67
Not applicable	92	61.3	5	3.3	104	69.33
Never	8	5.3	100	66.7	12	8
Total	150	100	150	100	150	100

Table 55: Quantities of kako often consumed at an instance by pregnant women

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
5-10	26	17.3	35	23.3	18	12
11-20	4	2.7	2	1.3	7	4.67
21-30	16	10.7	6	4	6	4
31-40	4	2.7	2	1.3	3	2
Not applicable	100	66.7	105	70	116	77.33
Total	150	100	150	100	150	100

Table 56: Frequency of consumption of koobi among pregnant women

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
Once a week	13	8.7	24	16	4	2.67
Once a month	45	30	26	17.3	42	28
Not applicable	92	61.3	100	66.7	104	69.33
Total	150	100	150	100	150	100

Table 57: Quantities of koobi often consumed at an instance by pregnant women

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
10-20	6	4	2	1.3	3	2
21-30	14	9.3	12	8	2	1.33
31-40	10	6.7	12	8	8	5.33
More than 40	28	18.7	24	16	33	22
Not applicable	92	61.3	100	66.7	104	69.33
Total	150	100	150	100	150	100

Table 58: Frequency of consumption of sundried sardines among pregnant women

	Jamestown		Tema New Town		Madina	
Frequency	Number	%	Number	%	Number	%
2-3 times a week	5	3.3	16	10.7	0	0
Once a week	34	22.7	27	18	12	8
Once a month	19	12.7	7	4.7	34	22.67
Not applicable	92	61.3	100	66.7	104	69.33
Total	150	100	150	100	150	100

Table 59: Quantities of sundried sardines often consumed at an instance by pregnant women

	Jamestown		Tema New Town		Madina	
Quantity (g)	Number	%	Number	%	Number	%
50-100	11	7.3	5	3.3	32	21.33
101-150	15	10	23	15.3	6	4
151-200	32	21.3	22	14.7	8	5.33
Not applicable	92	61.3	100	66.7	104	69.33
Total	150	100	150	100	150	100

Appendix 4: Microbiology of fish during traditional fish processing

I. Jamestown

Table 60: Microbiology of samples along tuna processing chain

		TPC				TCC				E. coli				
Sample		**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**LM**
Fresh tuna	S1	88	92	90	94	53	46	49.5	45	17	13	15	17	+
	S2	92	103	97.5		42	39	40.5		21	17	19		+
Fresh tuna	S1	72	89	80.5	83	30	37	33.5	31	11	14	12.5	16	+
after washing	S2	77	94	85.5		21	34	27.5		21	15	18		+
Water before	S1	39	41	40	44	22	27	24.5	26	15	11	13	15	+
washing	S2	44	53	48.5		25	31	28		13	19	16		+
Water after	S1	55	60	57.5	56	44	39	41.5	48	27	33	30	27	+
washing	S2	47	61	54		52	57	54.5		21	25	23		+
Tuna after	S1	0	0	0	0	0	0	0	0	0	0	0	0	-
smoking	S2	0	0	0		0	0	0		0	0	0		-

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^2 CFU/g or ml, ***E.coli*** x10^2 CFU/g or ml, **Av**: Average **G.Av**: Grand average C1-C2: counts for plate 1 and 2; S1- S2: samples 1 and 2

Table 61: Microbiology of samples along mackerel processing chain

		TPC				TCC				E. coli				
Sample		**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**LM**
Fresh mackerel	S1	0	0	0	0	0	0	0	0	0	0	0	0	-
	S2	0	0	0		0	0	0		0	0	0		-
Mackerel after	S1	5	9	7	6	3	0	2	3	2	1	2	2	+
washing	S2	7	0	4		5	1	3		1	1	1		+
Water before	S1	36	32	34	35	18	20	19	18	11	14	12.5	12	+
washing	S2	39	31	35		21	14	17.5		13	10	11.5		+
Water after	S1	44	36	40	43	20	27	23.5	26	21	27	24	24	+
washing	S2	51	42	46.5		23	33	28		19	30	24.5		+
Mackerel after	S1	0	0	0	0	0	0	0	0	0	0	0	0	-
smoking	S2	0	0	0		0	0	0		0	0	0		-

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^2 CFU/g or ml, ***E.coli*** x10^2 CFU/g or ml, **Av**: Average **G.Av**: Grand average C1-C2: counts for plate 1 and 2; S1- S2: samples 1 and 2

Table 62: Microbiology of samples along herrings processing chain

		TPC				TCC				E. coli				
Sample		**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**LM**
Fresh herrings	S1	0	0	0	0	0	0	0	0	0	0	0	0	-
	S2	0	0	0		0	0	0		0	0	0		-
Herrings after	S1	11	9	10	12	5	1	3	6	0	2	1	2	+
washing	S2	13	15	14		8	9	8.5		1	3	2		+
Water before	S1	39	34	36.5	31	13	15	14	14	9	5	7	8	+
washing	S2	21	28	24.5		17	11	14		10	7	8.5		+
Water after	S1	45	39	42	37	21	26	23.5	22	11	14	12.5	10	+
washing	S2	35	28	31.5		19	22	20.5		5	9	7		+
Herring after	S1	0	0	0	0	0	0	0	0	0	0	0	0	-
smoking	S2	0	0	0		0	0	0		0	0	0		-

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^2 CFU/g or ml, ***E.coli*** x10^2 CFU/g or ml, **Av**: Average **G.Av**: Grand average C1-C2: counts for plate 1 and 2; S1- S2: samples 1 and 2

II. Tema New Town

Table 63: Microbiology of samples along tuna processing chain

		TPC				TCC				E. coli				
Sample		**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**LM**
Fresh tuna	S1	122	111	117	113	71	69	70	64	21	27	24	24	+
	S2	113	106	110		64	53	58.5		23	25	24		+
Tuna after	S1	91	101	96	90	47	41	44	40	21	18	19.5	19	+
washing	S2	88	79	83.5		33	39	36		19	16	17.5		+
Water before	S1	31	35	33	33	31	28	29.5	30	20	25	22.5	24	+
washing	S2	29	37	33		37	23	30		26	21	23.5		+
Water after	S1	44	60	52	54	62	67	64.5	65	31	32	31.5	31	+
washing	S2	58	52	55		59	71	65		37	25	31		+
Tuna after	S1	0	0	0	0	0	0	0	0	0	0	0	0	-
smoking	S2	0	0	0		0	0	0		0	0	0		-

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^2 CFU/g or ml, ***E.coli*** x10^2 CFU/g or ml, **Av**: Average **G.Av**: Grand average C1-C2: counts for plate 1 and 2; S1- S2: samples 1 and 2

Table 64: Microbiology of samples along mackerel processing chain

		TPC				TCC				E. coli				
Sample		**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**LM**
Fresh mackerel	S1	0	0	0	0	0	0	0	0	0	0	0	0	+
	S2	0	0	0		0	0	0		0	0	0		+
Mackerel after washing	S1	16	10	13	12	15	8	11.5	13	7	4	5.5	6	+
	S2	11	9	10		17	12	14.5		8	3	5.5		+
Water before washing	S1	42	33	37.5	34	23	18	20.5	22	23	17	20	22	+
	S2	35	27	31		28	21	24.5		27	22	24.5		+
Water after washing	S1	48	63	55.5	55	40	47	43.5	45	31	43	37	33	+
	S2	57	53	55		43	51	47		28	30	29		+
Mackerel after smoking	S1	0	0	0	0	0	0	0	0	0	0	0	0	-
	S2	0	0	0		0	0	0		0	0	0		-

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^2 CFU/g or ml, ***E.coli*** x10^2 CFU/g or ml, **Av**: Average **G.Av**: Grand average C1-C2: counts for plate 1 and 2; S1- S2: samples 1 and 2

Table 65: Microbiology of samples along herrings processing chain

		TPC				TCC				E. coli				
Sample		**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**LM**
Fresh herrings	S1	0	0	0	0	0	0	0	0	0	0	0	0	-
	S2	0	0	0		0	0	0		0	0	0		-
Herrings after washing	S1	19	24	21.5	21	10	13	11.5	13	5	2	3.5	6	+
	S2	18	22	20		12	15	13.5		9	7	8		+
Water before washing	S1	39	32	35.5	30	21	25	23	21	15	11	13	12	+
	S2	21	27	24		18	21	19.5		10	12	11		+
Water after washing	S1	47	44	45.5	44	27	23	25	24	11	13	12	10	+
	S2	40	46	43		22	25	23.5		6	9	7.5		+
Mackerel after smoking	S1	0	0	0	0	0	0	0	0	0	0	0	0	-
	S2	0	0	0		0	0	0		0	0	0		-

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^2 CFU/g or ml, ***E.coli*** x10^2 CFU/g or ml, **Av**: Average **G.Av**: Grand average C1-C2: counts for plate 1 and 2; S1- S2: samples 1 and 2

Table 66: Microbiology of samples along kako processing chain

		TPC				TCC				E. coli				
Sample		**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**LM**
Fresh fish	S1	132	137	135	129	82	75	78.5	84	57	63	60	65	+
	S2	125	121	123		91	86	88.5		71	68	69.5		+
Fish after	S1	98	107	103	106	68	57	62.5	63	61	57	59	51	+
washing	S2	117	102	110		55	71	63		49	38	43.5		+
Water before	S1	64	50	57	59	38	35	36.5	33	25	18	21.5	22	+
washing	S2	58	65	61.5		27	32	29.5		21	23	22		+
Water after	S1	97	91	94	95	91	86	88.5	98	55	47	51	53	+
washing	S2	101	89	95		111	103	107		51	58	54.5		+
Fish after salting	S1	0	0	0	0	0	0	0	0	0	0	0	0	+
	S2	0	0	0		0	0	0		0	0	0		+
Fish after	S1	17	23	20	19	9	5	7	5	0	3	1.5	3	+
sundrying	S2	20	15	17.5		7	0	3.5		1	7	4		+

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^2 CFU/g or ml, ***E.coli*** x10^2 CFU/g or ml, **Av**: Average **G.Av**: Grand average C1-C2: counts for plate 1 and 2; S1- S2: samples 1 and 2

Table 67: Microbiology of samples along momoni processing chain

		TPC				TCC				E. coli				
Sample		**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**LM**
Fresh fish	S1	121	132	127	124	79	86	82.5	85	42	37	39.5	47	+
	S2	119	123	121		83	90	86.5		51	59	55		+
After washing	S1	108	111	110	119	77	54	65.5	63	38	26	32	29	+
	S2	131	127	129		63	59	61		29	22	25.5		+
Water before	S1	85	73	79	84	51	47	49	38	18	16	17	18	+
washing	S2	91	87	89		31	25	28		21	18	19.5		+
Water after	S1	161	142	152	151	79	62	70.5	73	19	13	16	23	+
washing	S2	155	147	151		82	71	76.5		32	27	29.5		+
Fish after salting	S1	0	0	0	0	0	0	0	0	0	0	0	0	+
and fermenting	S2	0	0	0		0	0	0		0	0	0		+
Fish after	S1	17	11	14	16.5	8	11	9.5	11	4	9	6.5	8	+
sundrying	S2	20	18	19		10	14	12		7	11	9		+

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^2 CFU/g or ml, ***E.coli*** x10^2 CFU/g or ml, **Av**: Average **G.Av**: Grand average C1-C2: counts for plate 1 and 2; S1- S2: samples 1 and 2

Table 68: Microbiology of samples along sundried fish processing chain

		TPC				TCC				E. coli				
Sample		**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**C1**	**C2**	**Av**	**G.Av**	**LM**
Fresh fish	S1	64	72	68	72	37	24	30.5	30	20	33	26.5	26	+
	S2	81	69	75		28	31	29.5		28	22	25		+
Fish after	S1	68	66	67	57	27	21	24	28	27	20	23.5	26	+
washing	S2	59	35	47		32	32	32		24	31	27.5		+
Water before	S1	28	21	24.5	31	17	12	14.5	18	5	11	8	5	+
washing	S2	41	32	36.5		19	21	20		0	2	1		+
Water after	S1	83	59	71	78	23	19	21	21	10	16	13	14	+
washing	S2	92	78	85		16	24	20		20	12	16		+
Fish after	S1	19	23	21	27	25	18	21.5	19	19	24	21.5	26	+
sundrying	S2	37	29	33		13	21	17		27	31	29		+

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^2 CFU/g or ml, ***E.coli*** x10^2 CFU/g or ml, **Av**: Average **G.Av**: Grand average C1-C2: counts for plate 1 and 2; S1- S2: samples 1 and 2

APPENDIX 5: OCCURRENCE OF *L. MONOCYTOGENES* AND OTHER MICROOGANISMS IN FISH ON INFORMAL MARKETS

Table 69: Microbial counts and detection of *Listeria monocytogenes* in traditionally processed fish purchased from Jamestown Market

Smoked Tuna																	
	TPC				**TCC**				***E. coli***				**LM**				**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	87	91	89		32	27	29.5		20	21	20.5		17	20	18.5		+
S2	71	69	70	83	24	22	23	23	19	17	18	19	15	11	13	12	+
S3	87	92	89.5		18	14	16		13	24	18.5		1	8	4.5		+
Smoked Mackerel																	
	TPC				**TCC**				***E. coli***				**LM**				**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	72	76	74		41	33	37		17	20	18.5		11	8	9.5		+
S2	74	68	71	75	58	44	51	40	22	19	20.5	18	6	6	6	9	+
S3	82	79	80.5		35	29	32		11	21	16		9	13	11		+
Smoked Herrings																	
	TPC				**TCC**				***E. coli***				**LM**				**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	85	79	82		25	25	25		20	21	20.5		14	23	18.5		+
S2	55	68	61.5	68	30	27	28.5	25	19	17	18	19	0	0	0	9	-
S3	63	59	61		21	19	20		13	24	18.5		5	11	8		+
Sundried Sardines																	
	TPC				**TCC**				***E. coli***				**LM**				**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	98	83	90.5		58	47	52.5		15	11	13		4	9	6.5		+
S2	75	67	71	74	50	45	47.5	47	17	21	19	18	0	0	0	2	-
S3	69	52	60.5		42	38	40		20	23	21.5		0	0	0		-

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^4 CFU/g or ml, ***E.coli*** x10^3 CFU/g or ml, **LM**: Listeria monocytogenes x10^2
Av: Average **G.Av**: Grand average **C1-C2**: counts for plate 1 and 2; **S1- S3**: samples 1,2,3 LM DET: detection of *Listeria monocytogenes*

Table 69 continued

Kako

	TPC				TCC				*E. coli*				LM				LM DET
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	22	29	25.5	26	19	21	20	20	10	13	11.5	9	4	2	3	1	+
S2	24	27	25.5		21	17	19		9	0	4.5		0	0	0		-
S3	31	25	28		20	23	21.5		10	10	10		0	0	0		-

Koobi

	TPC				TCC				E COLI				LM				LM DET
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	23	27	25	23	17	19	18	17	9	12	10.5	9	0	0	0	2	-
S2	19	25	22		11	16	13.5		0	7	3.5		4	9	6.5		+
S3	26	19	22.5		19	21	20		13	10	11.5		0	0	0		-

Momoni

	TPC				TCC				*E. coli*				LM				LM DET
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	37	29	33	27	21	23	22	20	0	3	1.5	6	1	0	0.5	2	+
S2	25	23	24		21	19	20		8	14	11		2	5	3.5		+
S3	27	22	24.5		16	21	18.5		0	11	5.5		0	0	0		-

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^4 CFU/g or ml, ***E.coli*** x10^3 CFU/g or ml, **LM**: Listeria monocytogenes x10^2 **Av**: Average **G.Av**: Grand average **C1-C2**: counts for plate 1 and 2; **S1- S3**: samples 1,2,3 LM DET: detection of *Listeria monocytogenes*

Table 70: Microbial counts and detection of *Listeria monocytogenes* in traditionally processed fish purchased from Tema New Town Market

Smoked Tuna																
	TPC				**TCC**				***E. coli***				**LM**			
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	75	61	68		45	37	41		18	22	20		0	0	0		-
S2	67	73	70	72	30	43	36.5	39	30	57	43.5	39	0	0	0	2	-
S3	80	73	76.5		39	33	36		47	60	53.5		5	8	7		+
Smoked Mackerel																
	TPC				**TCC**				***E. coli***				**LM**			
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	77	68	72.5		33	37	35		25	21	23		0	0	0		-
S2	70	83	76.5	79	41	61	51	43	31	27	29	28	5	7	6	4	+
S3	84	91	87.5		47	36	41.5		33	28	30.5		3	6	5		+
Smoked Herrings																
	TPC				**TCC**				***E. coli***				**LM**			
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	58	50	54		27	18	22.5		12	18	15		7	11	9		-
S2	61	53	57	50	31	27	29	23	14	11	12.5	17	0	0	0	3	-
S3	47	33	40		22	14	18		11	33	22		0	0	0		-
Sundried Sardines																
	TPC				**TCC**				***E. coli***				**LM**			
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	71	83	77		44	38	41		36	47	41.5		0	0	0		-
S2	89	92	90.5	80	51	47	49	43	32	28	30	33	5	7	6	3	+
S3	76	70	73		38	41	39.5		27	33	30		3	5	4		+

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^4 CFU/g or ml, ***E.coli*** x10^3 CFU/g or ml, **LM**: Listeria monocytogenes x10^2 **Av**: Average **G.Av**: Grand average **C1-C2**: counts for plate 1 and 2; **S1- S3**: samples 1,2,3 LM DET: detection of *L. monocytogenes*

Table 70 continued

Kako																
	TPC				**TCC**				**E COLI**				**LM**				**LM**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**DET**
S1	29	33	31		12	15	13.5		5	9	7		0	0	0		-
S2	26	29	27.5	28	17	22	19.5	14	0	0	0	2	0	0	0	1	-
S3	31	19	25		11	9	10		0	0	0		4	2	3		+
Koobi																	
	TPC				**TCC**				**E COLI**				**LM**				**LM**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**DET**
S1	12	9	10.5		0	0	0		0	0	0		0	0	0		-
S2	19	11	15	15	11	7	9	3	5	0	3	1	0	0	0	0	-
S3	21	17	19		0	0	0		0	0	0		0	0	0		-
Momoni																	
	TPC				**TCC**				**E COLI**				**LM**				**LM**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**DET**
S1	12	7	9.5		10	17	14		0	0	0		0	0	0		-
S2	17	15	16	15	15	22	19	11	0	0	0	0	0	0	0	0	-
S3	21	19	20		0	0	0		0	0	0		2	3	2		+

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^4 CFU/g or ml, ***E.coli*** x10^3 CFU/g or ml, **LM**: Listeria monocytogenes x10^2 **Av**: Average **G.Av**: Grand average **C1-C2**: counts for plate 1 and 2; **S1- S3**: samples 1,2,3 LM DET: detection of *Listeria monocytogenes*

Table 71: Microbial counts and detection of *Listeria monocytogenes* in traditionally processed fish purchased from Madina Market

Smoked Tuna																	
	TPC				**TCC**				***E. coli***				**LM**				**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	75	49	62		34	39	36.5		27	19	23		18	25	21.5		+
S2	101	86	93.5	87	42	36	39	39	21	24	22.5	25	27	32	29.5	21	+
S3	97	113	105		37	39	38		32	29	30.5		8	13	10.5		+
Smoked Mackerel																	
	TPC				**TCC**				***E. coli***				**LM**				**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	93	81	87		27	31	29		18	24	21		15	47	31		+
S2	73	77	75	76	33	37	35	30	27	31	29	24	33	51	42	29	+
S3	66	70	68		28	24	26		20	22	21		18	9	13.5		+
Smoked Herrings																	
	TPC				**TCC**				***E. coli***				**LM**				**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	36	42	39		31	29	30		17	25	21		14	10	12		+
S2	49	57	53	47	45	34	39.5	35	38	27	32.5	27	0	0	0	8	+
S3	51	49	50		37	32	34.5		23	31	27		18	7	12.5		+
Sundried Sardines																	
	TPC				**TCC**				***E. coli***				**LM**				**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	73	85	79		51	58	54.5		31	31	31		3	5	4		+
S2	80	69	74.5	76	44	39	41.5	52	28	35	31.5	31	1	7	4	3	+
S3	67	79	73		56	63	59.5		26	33	29.5		0	0	0		+

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^4 CFU/g or ml, ***E.coli*** x10^3 CFU/g or ml, **LM**: Listeria monocytogenes x10^2
Av: Average **G.Av**: Grand average **C1-C2**: counts for plate 1 and 2; **S1- S3**: samples 1,2,3 LM DET: detection of *Listeria monocytogenes*

Table 71 continued

Kako																
	TPC				**TCC**				***E. coli***				**LM**			**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	27	21	24		10	13	11.5		9	4	6.5		5	11	8		+
S2	28	23	25.5	26	18	10	14	12	3	10	6.5	7	6	18	12	7	+
S3	25	31	28		12	10	11		7	11	9		0	0	0		+
Koobi																	
	TPC				**TCC**				***E. coli***				**LM**			**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	19	20	19.5		11	14	12.5		3	3	3		15	19	17		+
S2	16	24	20	21	9	10	9.5	11	4	7	5.5	4	0	0	0	6	+
S3	25	21	23		13	11	12		3	4	3.5		0	0	0		-
Momoni																	
	TPC				**TCC**				***E. coli***				**LM**			**LM DET**
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	
S1	28	34	31		10	14	12		0	4	2		11	3	7		+
S2	51	46	48.5	43	13	19	16	13	1	3	2	3	1	4	2.5	3	+
S3	38	59	48.5		11	16	13.5		4	5	4.5		0	0	0		-

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^4 CFU/g or ml, ***E.coli*** x10^3 CFU/g or ml, **LM**: Listeria onocytogenes x10^2 **Av**: Average **G.Av**: Grand average **C1-C2**: counts for plate 1 and 2; **S1- S3**: samples 1,2,3 LM DET: +detection of *Listeria monocytogenes*

Table 72: Microbial counts and detection of *Listeria monocytogenes* in traditionally processed fish purchased from Kaneshie Market

Smoked Tuna																	
	SPC				**TCC**				**E COLI**				**LM**				
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	78	91	85		50	43	46.5		15	21	18		21	33	27		+
S2	92	102	97	89	61	55	58	49	20	17	19	20	16	11	14	17	+
S3	84	88	86		45	39	42		23	25	24		9	14	12		+
Smoked Mackerel																	
	SPC				**TCC**				**E COLI**				**LM**				
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	93	87	90		37	26	31.5		11	14	13		8	11	9.5		+
S2	76	73	75	86	38	31	34.5	35	24	24	24	22	3	0	1.5	16	+
S3	96	90	93		43	32	37.5		30	29	30		29	37	33		+
Smoked Herrings																	
	SPC				**TCC**				**E COLI**				**LM**				
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	76	79	78		30	34	32		20	23	22		0	0	0		-
S2	84	87	86	81	51	42	46.5	41	38	27	33	25	0	0	0	0	+
S3	79	83	81		48	39	43.5		19	22	21		0	0	0		+
Sundried Sardines																	
	SPC				**TCC**				**E COLI**				**LM**				
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	112	103	108		77	72	74.5		28	21	25		0	0	0		-
S2	90	97	94	97	34	28	31	50	24	19	22	25	0	0	0	0	+
S3	94	91	93		45	49	47		32	27	30		0	0	0		+

Table 72 continued

Kako																
	SPC				**TCC**				**E COLI**				**LM**			
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	67	53	60		23	20	21.5		13	7	10		0	0	0		-
S2	69	49	59	58	19	25	22	22	10	17	14	11	11	25	18	6	+
S3	55	57	56		21	27	24		9	11	10		0	0	0		-
Koobi																	
	SPC				**TCC**				**E COLI**				**LM**			
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	63	50	56.5		20	22	21		11	12	12		21	29	25		+
S2	51	63	57	57	19	26	22.5	20	9	10	9.5	11	0	0	0	9	-
S3	69	48	58.5		12	19	15.5		13	11	12		1	5	3		+
Momoni																	
	SPC				**TCC**				**E COLI**				**LM**			
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	30	37	33.5		17	15	16		10	12	11		19	12	16		+
S2	45	49	47	37	13	21	17	20	11	10	11	12	0	0	0	5	-
S3	29	31	30		23	28	25.5		14	12	13		0	0	0		+

TPC: Total plateTPC: Total plate count x105 CFU/g or ml, TCC: Total coliform x104 CFU/g or ml, E.coli x103 CFU/g or ml, LM: Listeria monocytogenes x102 Av: Average G.Av: Grand average C1-C2: counts for plate 1 and 2; S1- S3: samples 1,2,3 LM DET: detection of L. monocytogenes count x10^5 CFU/g or ml, **TCC**: Total coliform x10^4 CFU/g or ml, ***E.coli*** x10^3 CFU/g or ml, **LM**: Listeria monocytogenes x10^2 **Av**: Average **G.Av**: Grand average **C1-C2**: counts for plate 1 and 2; **S1- S3**: samples 1,2,3 LM DET: detection of *Listeria monocytogenes*

Table 73: Microbial counts and detection of *Listeria monocytogenes* in traditionally processed fish purchased from Agbogbloshie Market

	Smoked Tuna																
	SPC				TCC				E COLI				LM				LM DET
	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	
S1	79	73	76		31	28	30		35	27	31		10	14	12		+
S2	96	87	91.5	82	34	29	32	31	25	21	23	30	12	9	10.5	8	+
S3	82	77	79.5		29	33	31		32	40	36		0	0	0		-
	Smoked Mackerel																
	SPC				TCC				E COLI				LM				LM DET
	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	
S1	58	69	63.5		22	29	26		19	21`	19		5	3	4		+
S2	84	78	81	71	33	28	31	28	31	23	27	23	15	21	18	10	+
S3	61	73	67		34	21	28		20	25	23		7	11	9		+
	Smoked Herrings																
	SPC				TCC				E COLI				LM				LM DET
	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	
S1	47	32	39.5		52	47	50		21	18	20		3	1	2		+
S2	53	40	46.5	48	67	71	69	55	17	12	15	14	0	0	0	1	-
S3	61	53	57		55	38	47		10	8	9		1	0	0.5		+
	Sundried Sardines																
	SPC				TCC				E COLI				LM				LM DET
	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	C1	C2	Av.	G.Av	
S1	35	27	31		80	93	87		77	81	79		0	0	0		-
S2	50	44	47	42	97	103	100	89	72	93	83	80	0	0	0	0	-
S3	38	56	47		73	88	81		83	76	80		0	0	0		+

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^4 CFU/g or ml, ***E.coli*** x10^3 CFU/g or ml, **LM**: Listeria monocytogenes x10^2 **Av**: Average **G.Av**: Grand average **C1-C2**: counts for plate 1 and 2; **S1- S3**: samples 1,2,3 LM DET: detection of *Listeria monocytogenes*

Table 73 continued

Kako

	SPC				TCC				E COLI				LM				
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	27	31	29		12	7	9.5		0	0	0		2	3	2.5		+
S2	40	37	38.5	34	11	9	10	12	4	2	3	1	0	0	0	1	-
S3	38	32	35		15	19	17		0	0	0		1	0	0.5		+

Koobi

	SPC				TCC				E COLI				LM				
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	19	25	22		11	4	7.5		0	0	0		0	0	0		-
S2	22	17	19.5	24	7	16	12	10	0	0	0	0	0	0	0	0	-
S3	31	29	30		6	13	9.5		0	0	0		0	0	0		+

Momoni

	SPC				TCC				E COLI				LM				
	C1	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**C1**	**C2**	**Av.**	**G.Av**	**LM DET**
S1	21	23	22		9	8	8.5		0	0	0		0	0	0		-
S2	24	19	21.5	22	5	8	6.5	8	0	0	0	0	0	0	0	0	+
S3	28	20	24		12	4	8		0	0	0		0	0	0		-

TPC: Total plate count x10^5 CFU/g or ml, **TCC**: Total coliform x10^4 CFU/g or ml, ***E.coli*** x10^3 CFU/g or ml, **LM**: *Listeria monocytogenes* x10^2 **Av**: Average **G.Av**: Grand average **C1-C2**: counts for plate 1 and 2; **S1- S3**: samples 1,2,3 LM DET:

Appendix 6: Triangular distributions for probability of illness, P_{ill}, in Jamestown, Tema New Town and Madina

(a) Jamestown Respondents

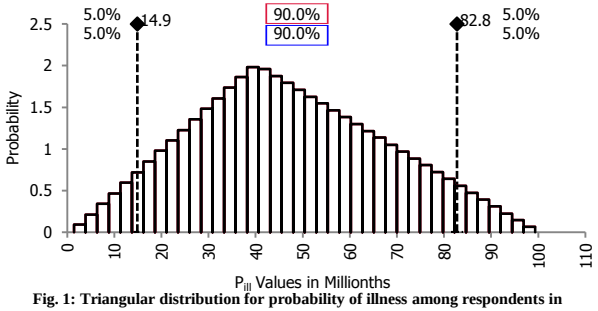

Fig. 1: Triangular distribution for probability of illness among respondents in Jamestown who consumed smoked tuna contaminated with *Listeria monocytogenes*

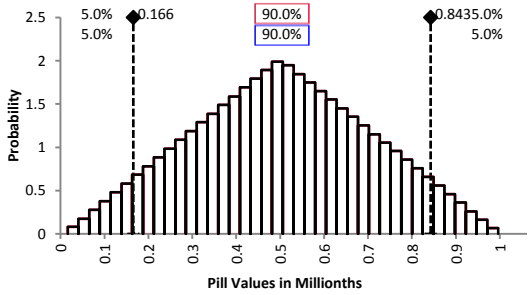

Fig. 4: Triangular distribution for probability of illness among respondents in Jamestown who consumed sundried sardines contaminated with Listeria monocytogenes

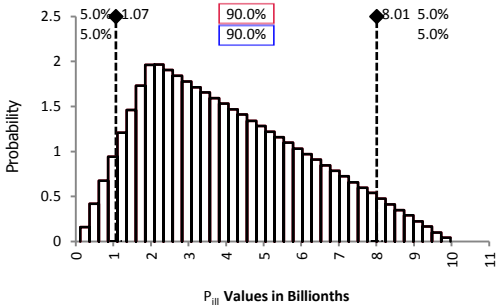

Fig. 7: Triangular distribution for probability of illness among respondents in Jamestown who consumed momoni contaminated with Listeria monocytogenes

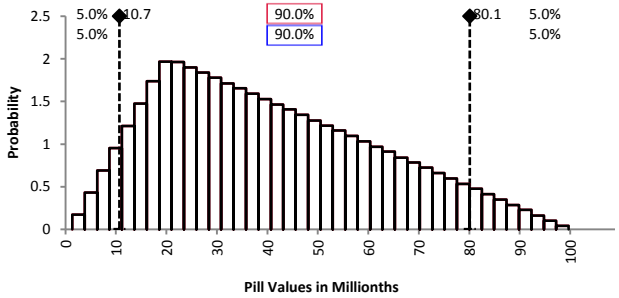

Fig. 2: Triangular distribution for probability of illness among respondents in Jamestown who consumed smoked mackerel contaminated with Listeria monocytogenes

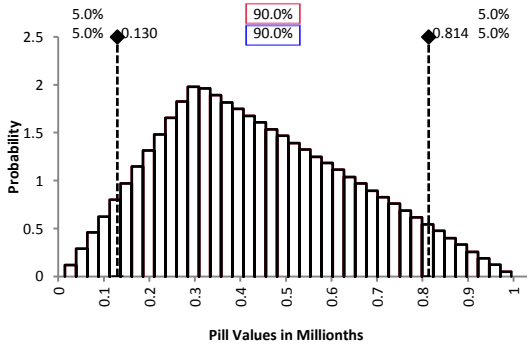

Fig. 5: Triangular distribution for probability of illness among respondents in Jamestown who consumed kako contaminated with Listeria monocytogenes

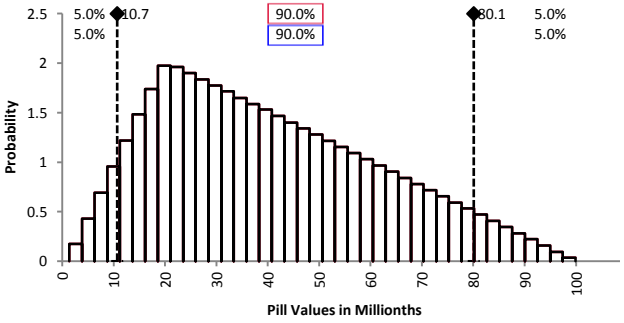

Fig 3: Triangular distribution for probability of illness among respondents in Jamestown who consumed smoked herrings contaminated with Listeria monocytogenes

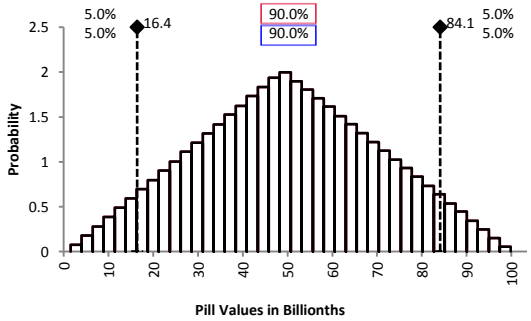

Fig.6: Triangular distribution for probability of illness among respondents in Jamestown who consumed koobi contaminated with Listeria monocytogenes

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(b) Jamestown Elderly

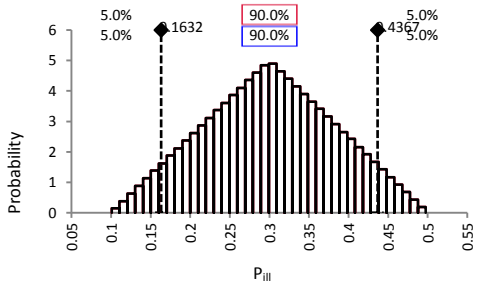

Fig. 8: Triangular distribution for probability of illness among the elderly in Jamestown who consume smoked tuna contaminate d with Listeria monocytogenes

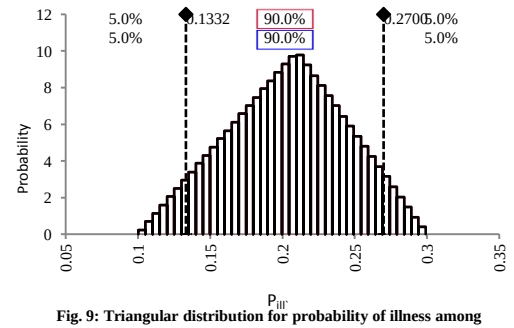

Fig. 9: Triangular distribution for probability of illness among the elderly in Jamestown who consume smoked mackerel contaminated with Listeria monocytogenes

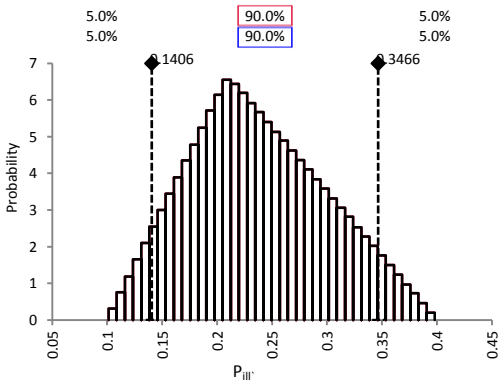

Fig. 10: Triangular distribution for probability of illness among the elderly in Jamestown who consume smoked herrings contaminated with Listeria monocytogenes

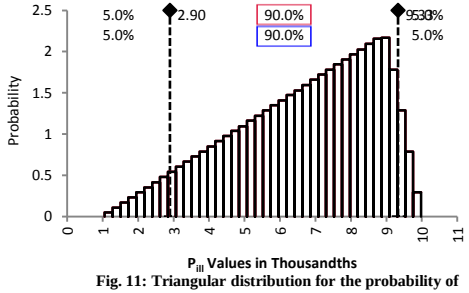

Fig. 11: Triangular distribution for the probability of illness among the elderly in Jamestown who consume sundried sardines contaminated with Listeria monocytogenes

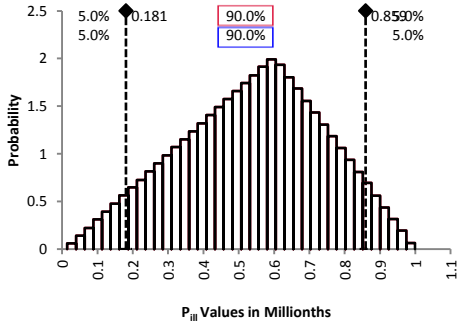

Fig. 12:Triangular distribution for the probability of illness among the elderly in Jamestown who consume kako contaminated with Listeria monocytogenes

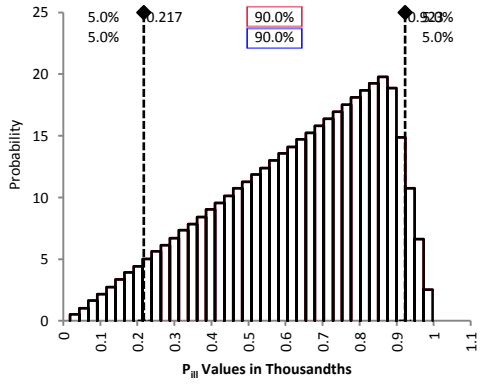

Fig. 13: Triangular distribution for the probability of illness among the elderly in Jamestown who consume koobi contaminated with Listeria monocytogenes

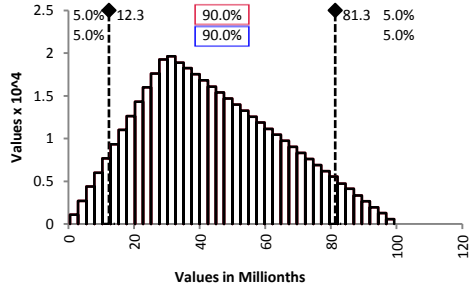

Fig. 14: Triangular distribution for the probability of illness among the elderly in Jamestown who consume momoni contaminated with Listeria monocytogenes

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(c) Jamestown Children

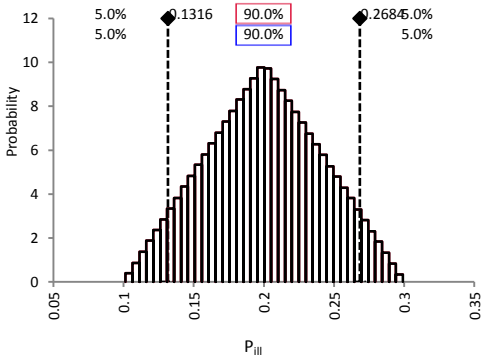


Fig. 15: Triangular distribution for probability of illness among children in Jamestown who ingest Listeria monocytogenes through consumption of smoked tuna

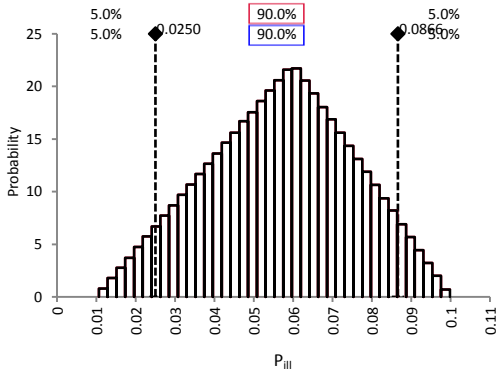


Fig. 17: Triangular distribution for probability of illness among children in Jamestown ingesting Listeria monocytogenes through consumption of smoked herrings

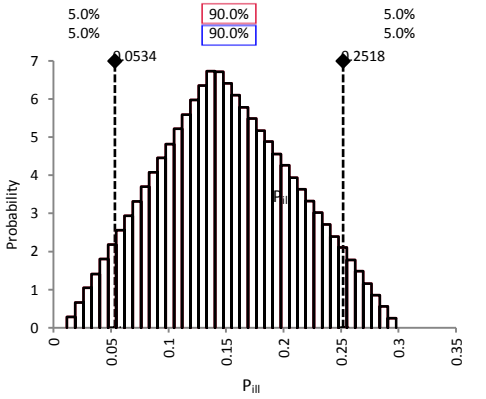


Fig. 16: Triangular distribution for probability of illness among children in Jamestown ingesting Listeria monocytogenes through consumption of smoked mackerel

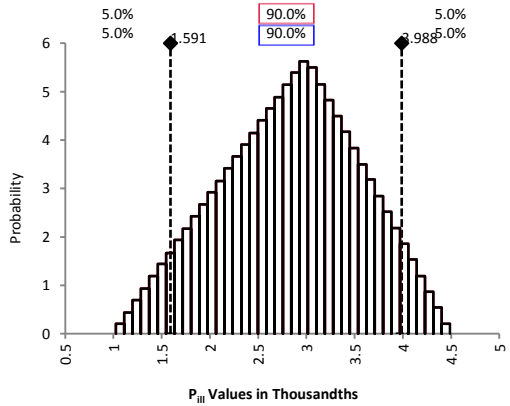


Fig. 18: Triangular distribution for probability of illness among children in Jamestown ingesting Listeria monocytogenes through consumption of sundried sardines

(d) Jamestown Pregnant Women

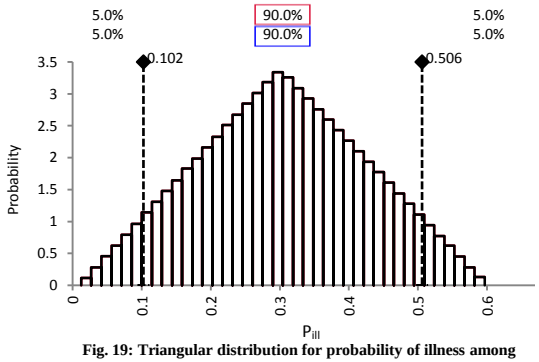


Fig. 19: Triangular distribution for probability of illness among pregnant women in Jamestown who consumed smoked tuna contaminated with Listeria monocytogenes

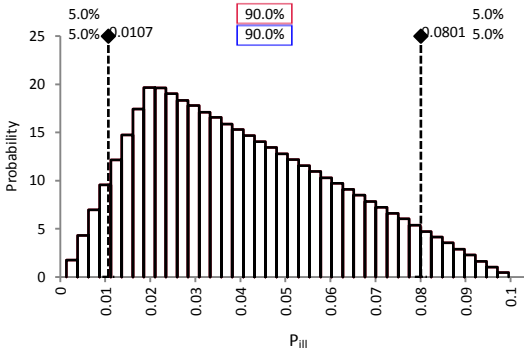


Fig. 22: Triangular distribution for probability of illness among pregnant women in Jamestown who consumed sundried sardines contaminated with Listeria monocytogenes

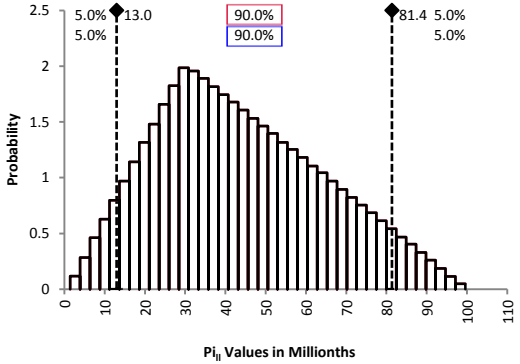


Fig 25: Triangular distribution for probability of illness among pregnant women in Jamestown who consumed momoni contaminated with Listeria monocytogenes

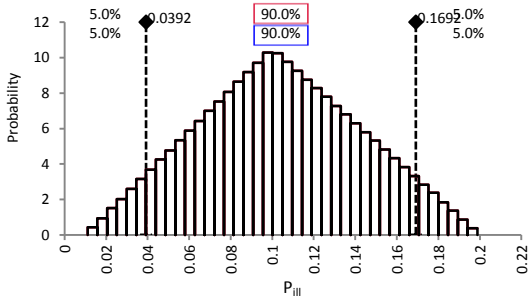


Fig 20" Triangular distribution for probability of illness among pregnant women in Jamestown who consumed smoked mackerel contaminated with Listeria monocytogenes

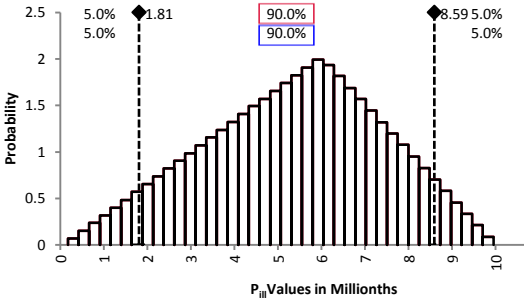


Fig. 23: Triangular distribution for probability of illness a pregnant women in Jamestown who consumed kako contaminated with Listeria monocytogenes

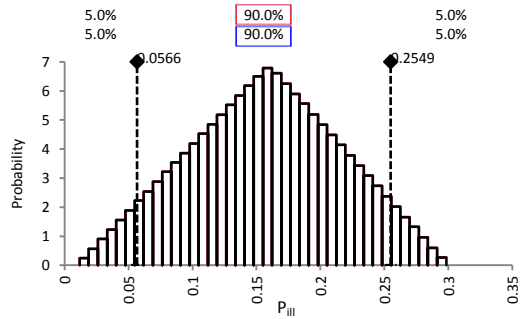


Fig. 21: Triangular distribution for probability of illness among pregnant women in Jamestown who consumed smoked herrings contaminated with Listeria monocytogenes

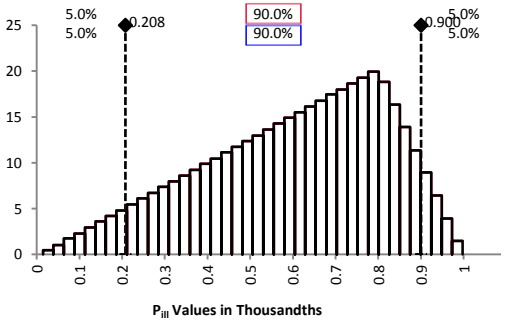


Fig. 24: Triangular distribution for probability of illness among pregnant women in Jamestown who consumed koobi contaminated with Listeria monocytogenes

(e) Tema New Town Respondents

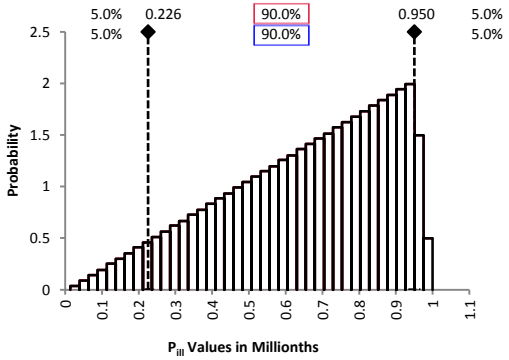


Fig. 26: Triangular distribution for probability of illness among respondents in Tema New Town who consumed smoked tuna contaminated with Listeria monocytogenes

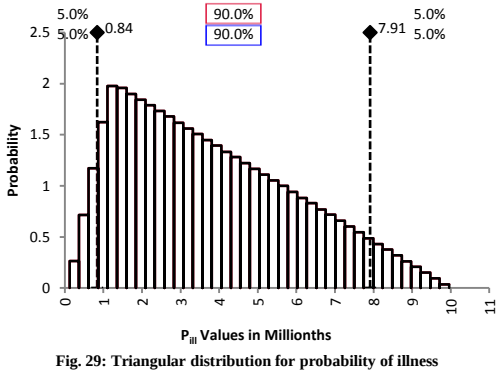


Fig. 29: Triangular distribution for probability of illness among respondents in Tema New Town who consumed sundried sardines contaminated with Listeria monocytogenes

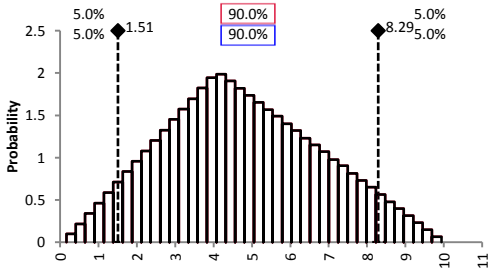


Fig. 2Triangular distribution for probability of illness among respondents in Tema New Town who consumed smoked mackerel contaminated with Listeria monocytogenes

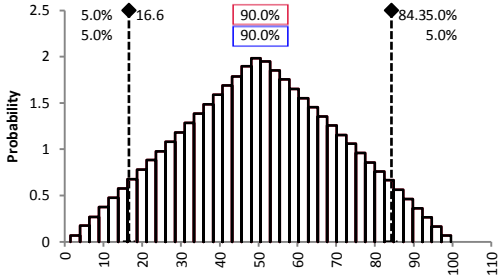


Fig. 30: Triangular distribution for probability of illness among respondents in Tema New Town who consumed kako contaminated with Listeria monocytogenes

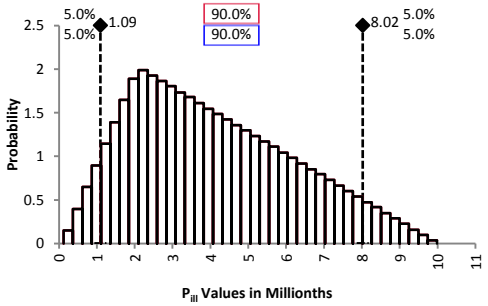


Fig. 28: Triangular distribution for probability of illness among respondents in Tema New Town who consumed smoked herrings contaminated with Listeria monocytogenes

(f) Tema New Town Elderly

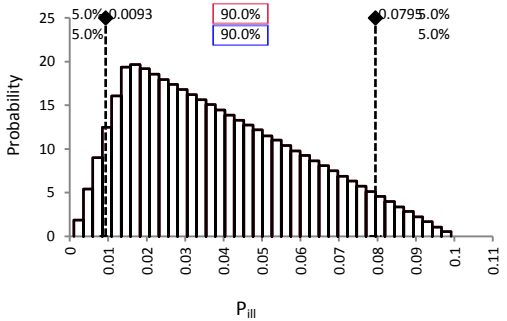


Fig. 31: Triangular distribution for probability of illness among the elderly in Jamestown who consumed smoked tuna contaminated with Listeria monocytogenes

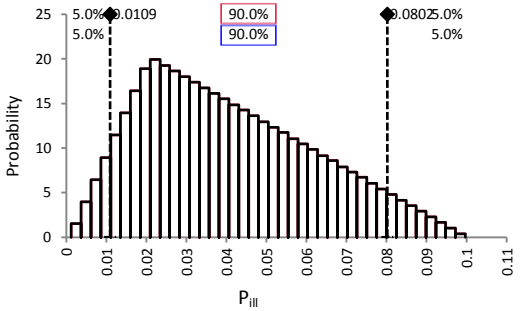


Fig. 34: Triangular distribution for probability of illness among the elderly in Jamestown who consumed sundried sardines contaminated with Listeria monocytogenes

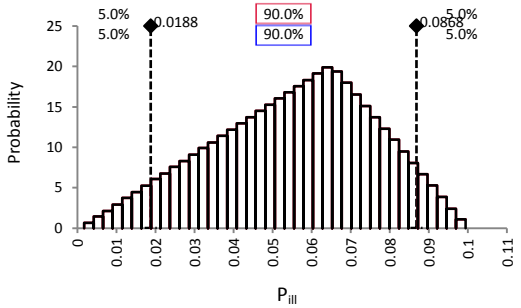


Fig. 32: Triangular distribution for probability of illness among the elderly in Jamestown who consumed smoked mackerel contaminated with Listeria monocytogenes

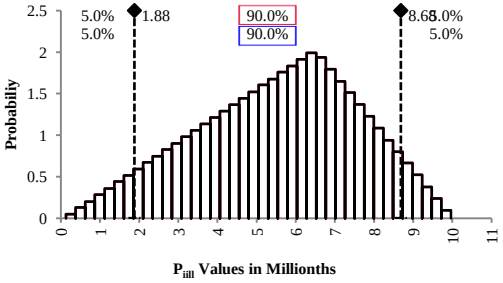


Fig. 35: Triangular distribution for probability of illness among the elderly in Jamestown who consumed kako contaminated with Listeria monocytogenes

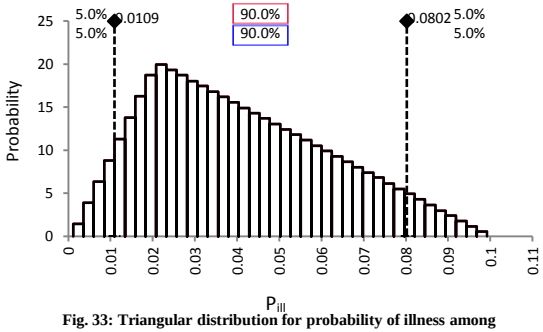


Fig. 33: Triangular distribution for probability of illness among the elderly in Jamestown who consumed smoked herrings contaminated with Listeria monocytogenes

(g) Tema New Town Children

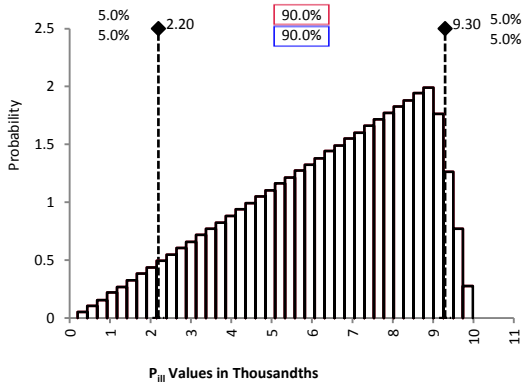
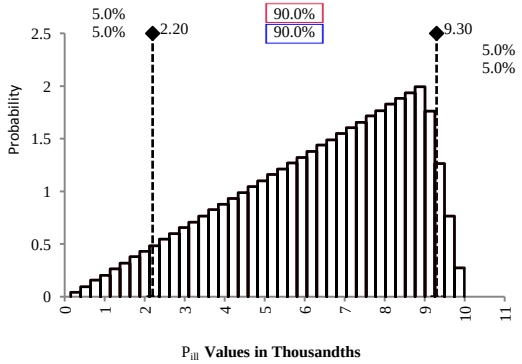


Fig. 36: Triangular distribution for probability of illness among children in Jamestown who consumed smoked tuna contaminated with Listeria monocytogenes



Fi.g 38: Triangular distribution for probability of illness among children in Jamestown who consumed smoked herrings contaminated with Listeria monocytogenes

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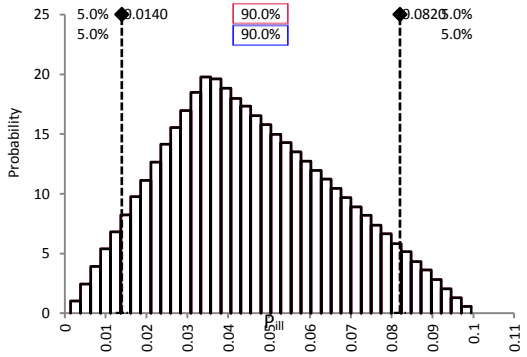

Fig. 37: Triangular distribution for probability of illness among children in Jamestown who consumed smoked mackerel contaminated with Listeria monocytogenes

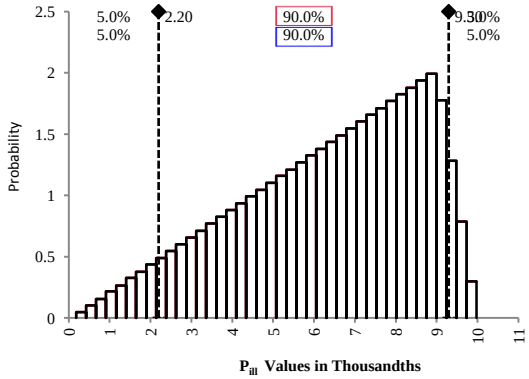

Fig. 39: Triangular distribution for probability of illness among children in Jamestown who consumed sundried sardines contaminated with Listeria monocytogenes

(h) Tema New Town Pregnant Women

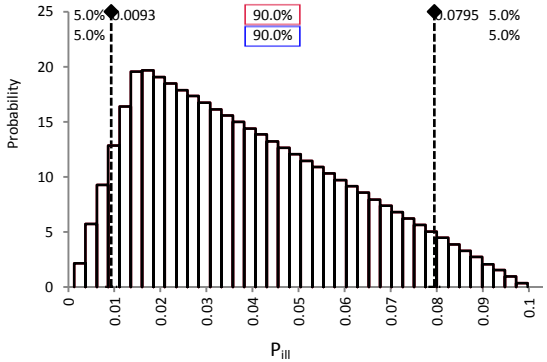

Fig. 40: Triangular distribution for probability of illness among pregnant women in Tema New Town who consumed smoked tuna contaminated with Listeria monocytogenes

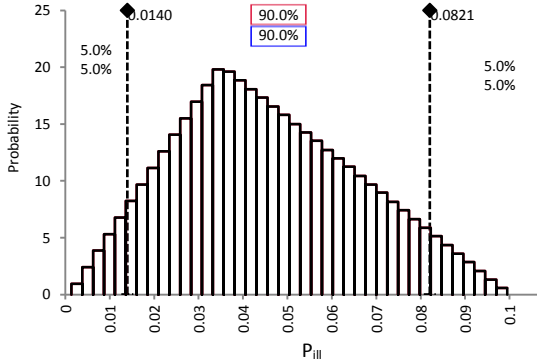

Fig. 43: Triangular distribution for probability of illness among pregnant women in Tema New Town who consumed sundried sardines contaminated with Listeria monocytogenes

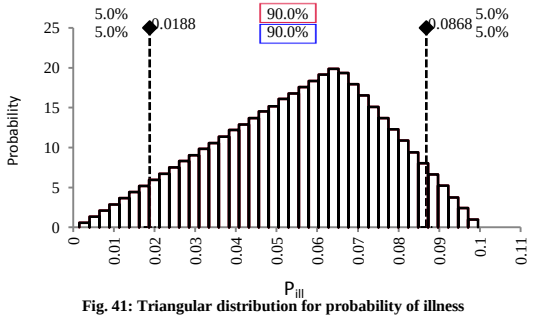

Fig. 41: Triangular distribution for probability of illness among pregnant women in Tema New Town who consumed smoked mackerel contaminated with Listeria monocytogenes

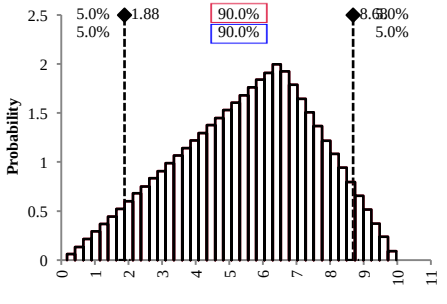

Fig. 44: Triangular distribution for probability of illness among pregnant women in Tema New Town who consumed kako contaminated with Listeria monocytogenes

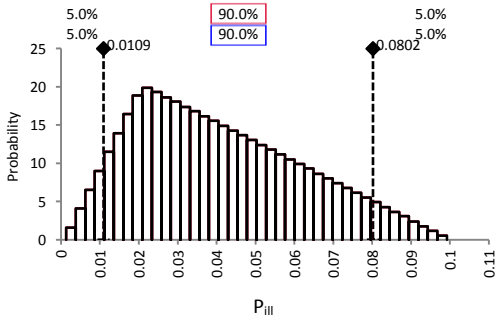

Fig. 42: Triangular distribution for probability of illness among pregnant women in Tema New Town who consumed smoked herrings contaminated with Listeria monocytogenes

(i) Madina Respondents

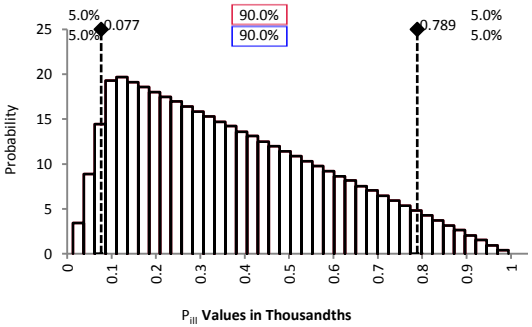

Fig. 45: Triangular distribution for probability of illness among respondents in Madina who consumed smoked salmon contaminated with Listeria monocytogenes

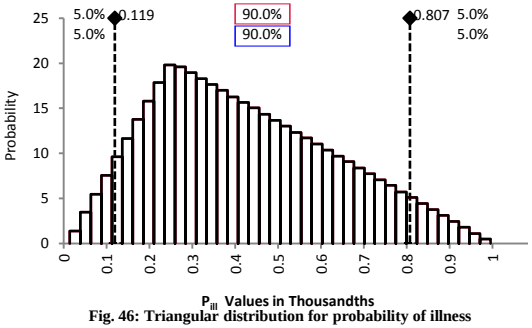

Fig. 46: Triangular distribution for probability of illness among respondents in Madina who consumed smoked mackerel contaminated with Listeria monocytogenes

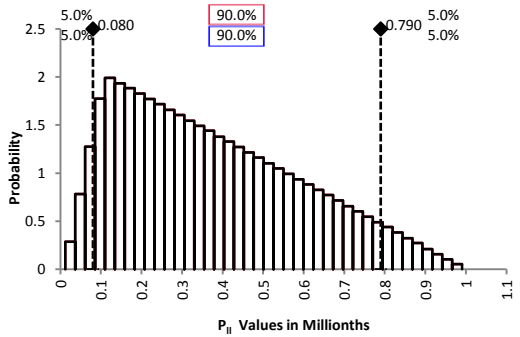

Fig. 47: Triangular distribution for probability of illness among respondents in Madina who consumed smoked herrings contaminated with Listeria monocytogenes

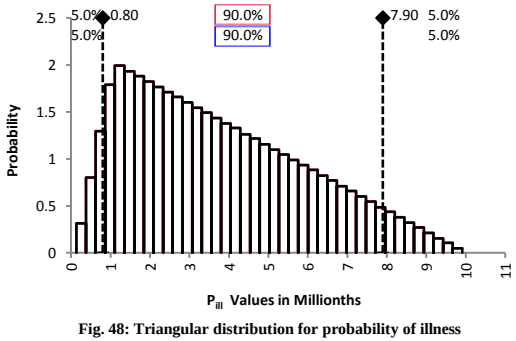

Fig. 48: Triangular distribution for probability of illness among respondents in Madina who consumed sundried sardines contaminated with Listeria monocytogenes

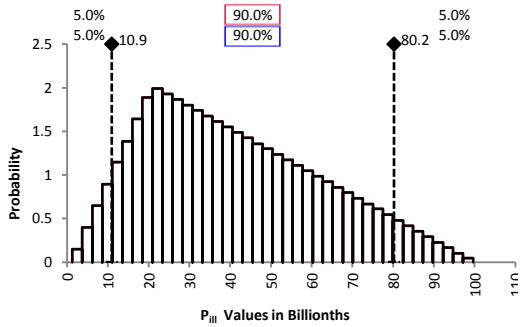

Fig. 49: Triangular distribution for probability of illness among respondents in Madina who consumed kako contaminated with Listeria monocytogenes

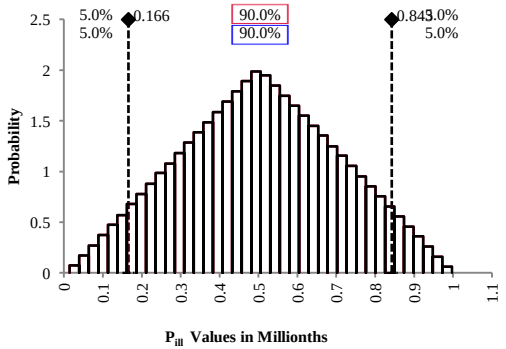

Fig. 50: Triangular distribution for probability of illness among respondents in Madina who consumed koobi contaminated with Listeria monocytogenes

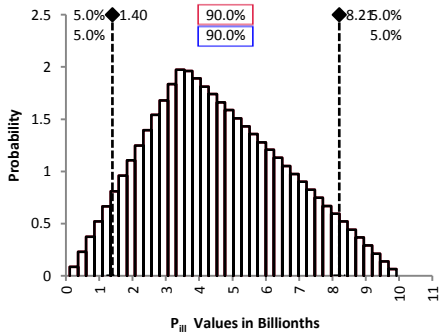

Fig. 51: Triangular distribution for probability of illness among respondents in Madina who consumed momoni contaminated with Listeria monocytogenes

(j) Madina Elderly

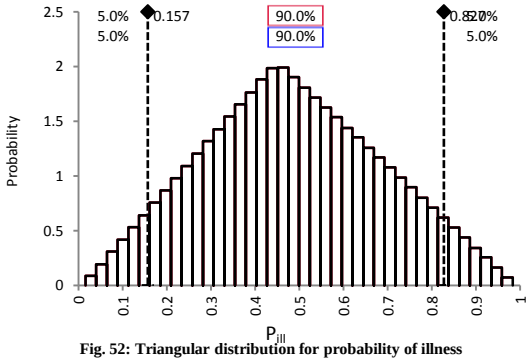

Fig. 52: Triangular distribution for probability of illness among the elderly in Madina who consumed smoked tuna contaminated with Listeria monocytogenes

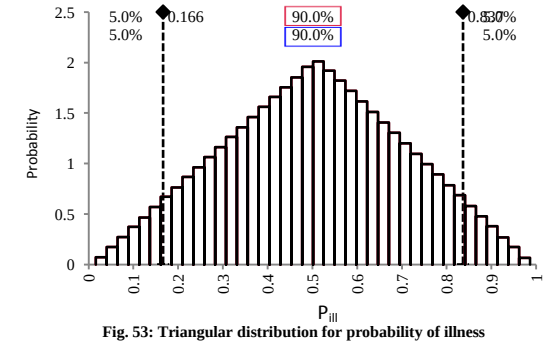

Fig. 53: Triangular distribution for probability of illness among the elderly in Madina who consumed smoked mackerel contaminated with Listeria monocytogenes

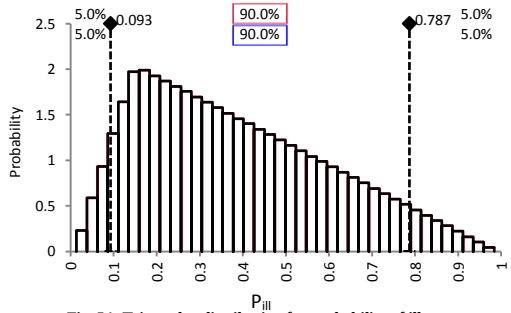

Fig. 54: Triangular distribution for probability of illness among the elderly in Madina who consumed smoked herrings contaminated with Listeria monocytogenes

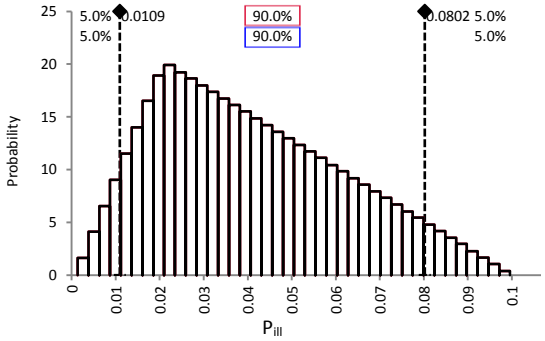

Fig. 55: Triangular distribution for probability of illness among the elderly in Madina who consumed sundried sardines contaminated with Listeria monocytogenes

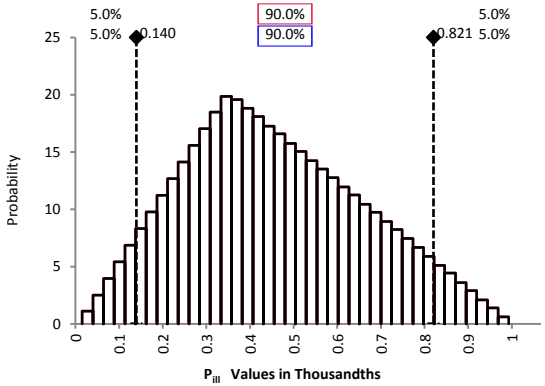

Fig. 56: Triangular distribution for probability of illness among the elderly in Madina who consumed kako contaminated with Listeria monocytogenes

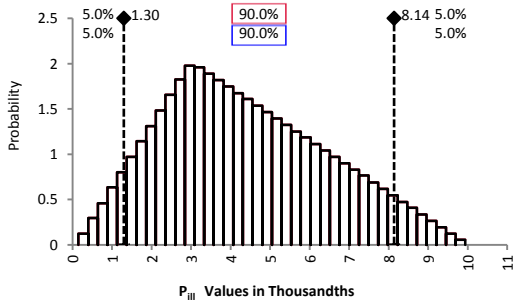

Fig. 57: Triangular distribution for probability of illness among the elderly in Madina who consumed koobi contaminated with Listeria monocytogenes

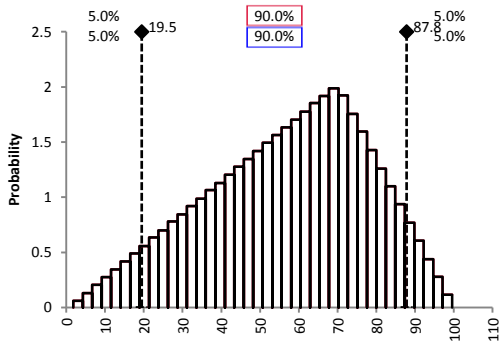

Fig. 58: Triangular distribution for probability of illness among the elderly in Madina who consumed momoni contaminated with Listeria monocytogenes

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(k) Madina Children

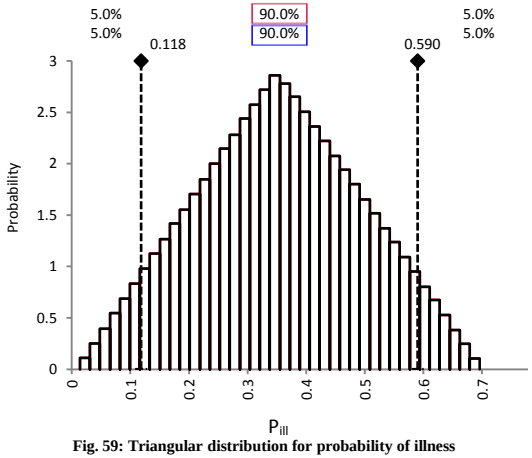

Fig. 59: Triangular distribution for probability of illness among children in Madina who consumed smoked tuna contaminated with Listeria monocytogenes

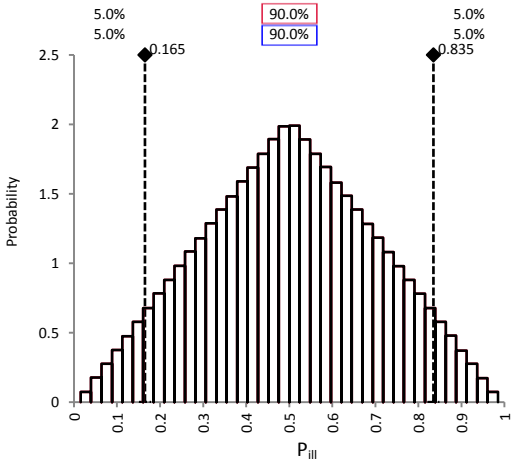

Fig. 61: Triangular distribution for probability of illness among children in Madina who consumed smoked mackerel contaminated with Listeria monocytogenes

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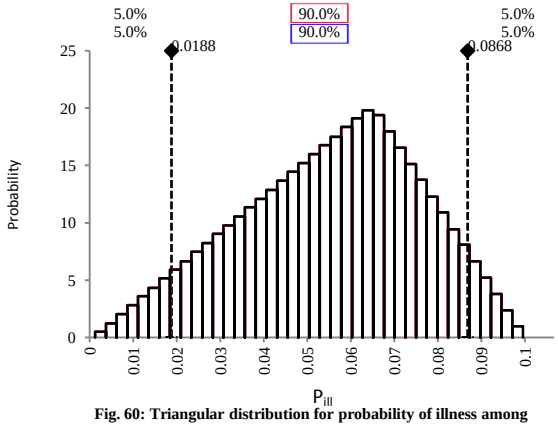

Fig. 60: Triangular distribution for probability of illness among children in Madina who consumed smoked herring contaminated with Listeria monocytogenes

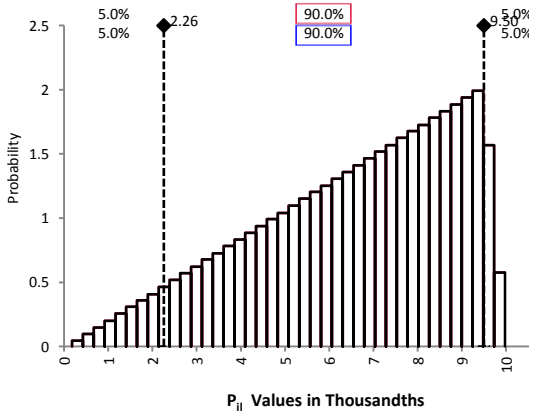

Fig. 62: Triangular distribution for probability of illness among children in Madina who consumed sundried sardines contaminated with Listeria monocytogenes

(l) Madina Pregnant Women

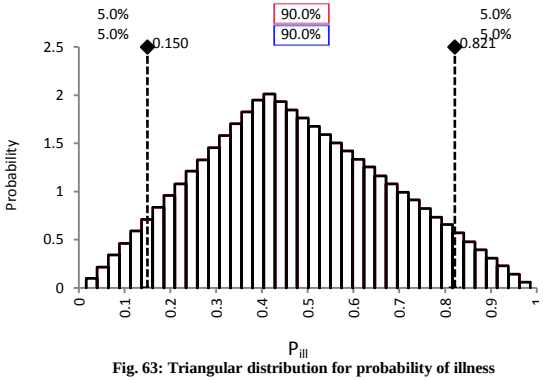

Fig. 63: Triangular distribution for probability of illness among pregnant women in Madina who consumed smoked tuna contaminated with Listeria monocytogenes

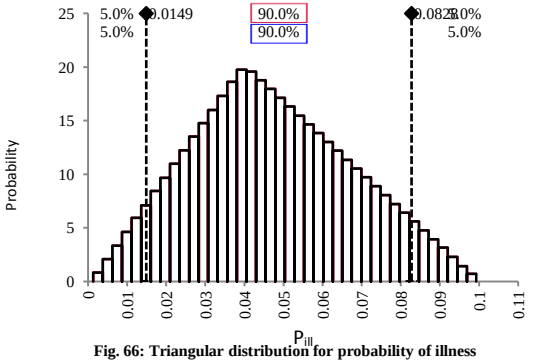

Fig. 66: Triangular distribution for probability of illness among pregnant women in Madina who consumed sundried sardines contaminated with Listeria monocytogenes

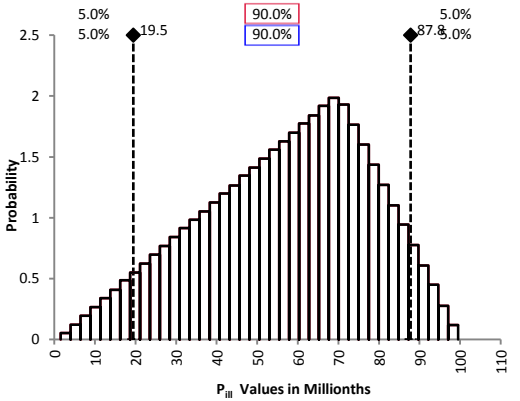

Fig. 69: Triangular distribution for probability of illness among pregnant women in Madina who consumed momoni contaminated with Listeria monocytogenes

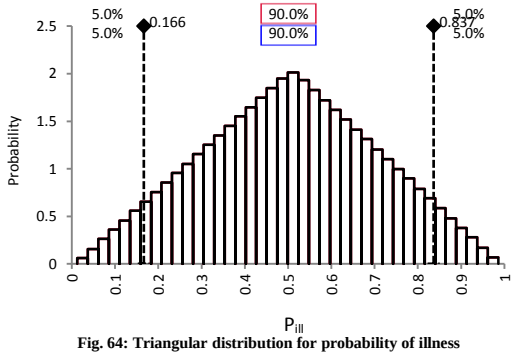

Fig. 64: Triangular distribution for probability of illness among pregnant women in Madina who consumed smoked mackerel contaminated with Listeria monocytogenes

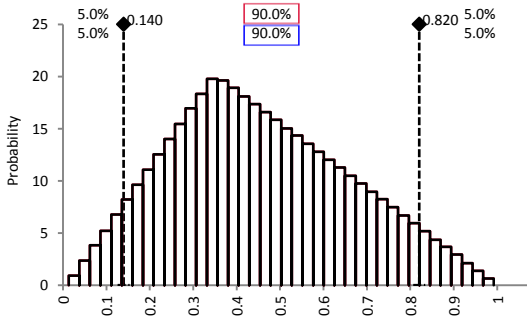

Fig. 67: Values in Thousandths
Triangular distribution for probability of illness among pregnant women in Madina who consumed kako contaminated with Listeria monocytogenes

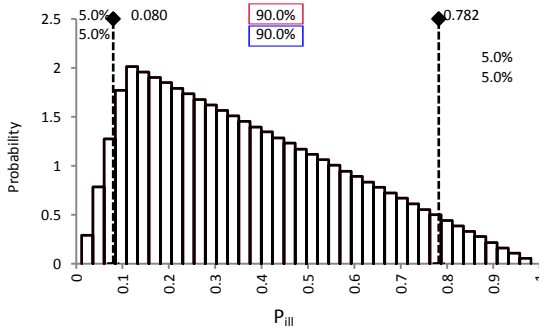

Fig. 65: Triangular distribution for probability of illness among pregnant women in Madina who consumed smoked herrings contaminated with Listeria monocytogenes

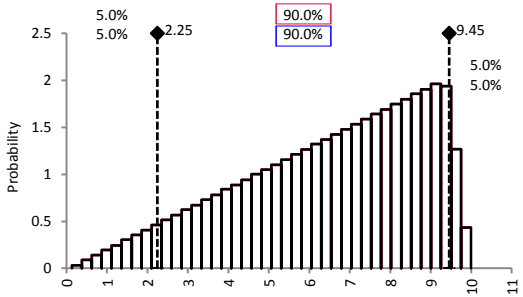

Fig. 68: Triangular distribution for probability of illness among pregnant women in Madina who consumed koobi contaminated with Listeria monocytogenes

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